Transcript

Sixth Meeting of the Secretary's Advisory Committee on Xenotransplantation

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Agenda Item: Welcome and Opening Remarks

DR. VANDERPOOL: Let us begin. I welcome each and all of you to this, the sixth open—full open meeting of the Secretary's Advisory Committee for Xenotransplantation that we will call SACX all day long. Welcome, of course, the full members of SACX. It's great to see all of you again, and to each and all our speakers and presenters, I—including our special speakers from Mexico and Canada, certainly to all our nonvoting agency representatives, without whom we would lose our way from time to time, and also to members of the public.

As you know, this is a public meeting, and part of our task is to educate the public about these important issues involving health and safety, and so we welcome you, and are open to discussing our issues with you. There will be times in the program when public comments are especially welcome, and you can do that from the mike that is—the microphone that is available.

We will, I understand, probably a little later in the day, have a Canadian film crew who is doing a documentary on xenotransplantation research of Dr. Valdes and others, and we welcome them to do their work. I trust that, as promised, that they will remain as unobtrusive as possible, but will not, nevertheless keep some of us from being able to appear in a movie.

A word about the background of what this Advisory Committee has been about. We began almost exactly three years ago, February of 2001, at which time we surveyed the ethical and scientific and social and psychological issues pertinent to xenotransplantation in order to begin to bring ourselves up to speed with respect to all of these issues pertaining to xenotransplantation. By the fall of that year, we'd already thought and even initiated efforts to put together two reports for the Secretary of the Department of Health and Human Services, namely one on informed consent, and one on the state of the science. And so in a rather consistent way, throughout 2002 and 2003, we worked on those reports sometimes in break-out sessions from the full meeting, and sometimes a meeting individually as the two subgroups on the committee worked on their respective reports. Our fourth meeting in March of 2002 was wholly devoted and called a science symposium, in which we sought to arrive at the best up-to-date understanding of the science of xeno that we could.

In November of 2002, Robyn Shapiro and I made a presentation about our work to the annual Prim&R meeting of Public Responsibility in Medicine and Research on the West Coast. Our last, fifth meeting, was held about a year ago, and so our being together today is, among other things, has some feelings of a class reunion.

After this meeting, some of our members, several of our members, will be rotating off the committee, all of whom we will greatly miss, and we certainly thank you deeply for your varied and essential contributions.

Now the day before us is interesting and full. We begin with updates from federal agencies, and then we will focus on our two reports. First we will deal with the report on informed consent in xeno and clinical research involving xenotransplantation, and my ardent hope is that the full committee will be able to vote to accept and endorse that report, which means that we would vote that it would be published for public comment. And then with respect to the State of the Science Report, I truly hope that we can arrive at a full and clear set of recommendations that would enable this report to move forward toward acceptance and publication in the very near future.

In an important respect, the entire meeting will contribute to the acceptance of these reports, because after our agency updates, and after we critically review the informed consent report, we will hear a number of presentations, on the science of xeno, presentations from Drs. Cooper, Sachs, Salomon, Sykes, and Patience, and then we will hear about the clinical trial that is being conducted in Mexico, Mexico City. And then with these presentations in mind, we will move at the end of the day, in a lengthy session to an overview and critical evaluation of the State of the Science Report that will—may well be informed by each of these presentations.

So with that overview, let us proceed, and I shall turn to Dr. Mary Groesch, our able Executive Director, for further comments and some housekeeping activities, I guess we could call them institutional-keeping activities, now.

DR. GROESCH: Thank you, Harold. I would like to give just a brief overview of the rules of conduct and conflict of interests, similar to what I have done in the past. As members of the U.S. Department of Health and Human Services Secretary's Advisory Committee on Xenotransplantation, you are special government employees, and are

therefore subject to rules of conduct that apply to government employees. These rules and regulations are explained in a report titled "Standards of Ethical Conduct for Employees of the Executive Branch." You each received a copy of this document when you were appointed to the committee.

At every meeting, in addition to reminding you about the importance of following the ethics rules, we always like to review the steps that we take, and ask you to take to ensure that any conflicts of interest between your public responsibilities and your private interests and activities are identified and addressed. As you know, before every meeting, you provide us with information about your personal, professional, and financial interests. We use this information as the basis for assessing whether you have any real, potential, or apparent conflicts of interests that could compromise your ability to be objective in giving advice during committee meetings.

If such conflicts are identified, we either issue a waiver, or recuse you from a particular portion of the meeting. We usually waive conflicts of interest for general matters, because we believe your ability to be objective will not be affected by your interests in such matters.

We also rely, to a great degree, on you to be attentive during our meetings to the possibility that an issue arises that could affect, or appear to affect, your interests in a specific way, and if this happens, we ask you to recuse yourself from the discussions.

If you have any questions about rules of conduct or conflict of interests, our new committee management officer, Miss Carolyn Baum, is here this morning, and she'll be happy to address them at the first break.

And we're also pleased to have another expert, Miss Nancy Middendorf, here this morning. Nancy is a senior program analyst from the NIH Office of Federal Advisory Committee Policy, and we look to her frequently for wisdom and guidance on committee management issues.
And right now Carolyn Baum is going to tell us very briefly about a new conflict of interest procedure that is being implemented.

MS. BAUM: Good morning. I'm Carolyn Baum. All of you had received communications from me, and I'd like to say thank you for your very quick response to all of the documents that we asked you to fill out. Today on your table you'll find a red folder. This is marked "Confidential," and it is individualized for you as a committee member. The information it contains is based on the documentation that you forwarded to us, your updates on your conflicts of financial interest. We are asking that you, toward the end of the meeting, sign the certification that is enclosed, and if you have any further questions about the recusal list or the certification, again, I will be here at the break. Thank you again for your cooperation.

DR. GROESCH: Thank you, Carolyn.

Okay, our first agenda item, is meeting and activity updates, and our first speaker is Dr. Ellen Gadbois from the Department in the Office of the Secretary with the Office of the Assistant Secretary for Planning and Evaluations. Ellen.

Agenda Item: Meeting/Activity Updates

DR. GADBOIS: Thanks, Mary. On behalf of the Secretary, and also on behalf of Dr. Zucker, the Deputy Assistant Secretary for Health, who will be arriving later this morning, I just wanted to welcome you all, and thank you for your past years of service on this committee.
In particular, we wanted to thank Dr. Vanderpool and Dr. Groesch for their able leadership of this group, and we look forward to receiving their two reports when we are finished.

As we've discussed in the past, the Secretary is very concerned about the shortage of organs and tissues in this country, and xenotransplantation obviously has the potential to help alleviate this shortage. We also have to balance the needs of patients with possible risks from zoonotic diseases, and your work has been greatly beneficial in helping the department think about these issues. And we anticipate that your reports will also assist us as we continue forward in this area.

Dr. Zucker, at the last meeting, mentioned that we were going to go forward with some activities internationally. In December, the State Department issued a demarche to all of our embassies worldwide, asking them to contact the Ministries of Health in their countries, and raise the issues of xenotransplantation that you are all very familiar with.

We specifically asked the embassies to report back to us on activities going on in those countries in xenotransplantation, and in particular, whether they had knowledge of U.S. citizens traveling abroad to undergo xenotransplantation. We are just beginning to get feedback from our embassies, and so as we get a few more of those, we'll be meeting with our colleagues at the State Department, and discussing whether there are any particular actions we should be taking.
We've also begun a new effort with the World Health Organization, and Dr. Bloom will be telling you more about that in a moment, but we intend to stay in contact with the WHO about the information we received in response to the demarches, and continue working with our international partners in this area trying to raise awareness of the issues, and get better information about what is going on worldwide.

So with that, thanks again. We look forward to hearing the discussion today, and in particular, we want to welcome our two international visitors, Dr. Valdes and Dr. White. Thank you.

DR. GROESCH: Thank you, Ellen. Our next speaker is Dr. Tom Spira from the Centers for Disease Control and Prevention, and he will be talking to us about a national biologic archive for xenotransplantation.

DR. SPIRA: Good morning. It is a pleasure being back with this group. I remember being here three or four years ago at the first meeting of this group. At that time, things were not as developed, and we had a lot of plans. The Department's guidelines for xenotransplantation had come out in 1996, and it was finalized and issued in the year 2001. As part of that activity, CDC took on a major role of setting up a biological archive for xenotransplantation specimens for use in public health purposes. That is what I'd like to talk about today.

I'm going to quote, just to give you some background, from the guidelines document. In Section 5.2, regarding the biological specimen archives, it said, "The sponsor should ensure that the designated PHS specimens from source animals, xenotransplantation products, and xenotransplantation product recipients, are archived. The biological specimen should be collected and archived under conditions that will ensure their suitability for subsequent public health purposes, including public health investigations.

"The location and nature of archived specimens should be documented in the health care records, and this information should be linked to the National Xenotransplantation Database when the latter becomes functional." DHHS was, at that time, considering options for a central biological archive, one which would be maintained either by private-sector organizations or contracted through DHHS. "These designated PHS specimens would then be deposited in such an archive. When feasible, a biopsy of the non-human animal live cells, tissues, or organs intended for use in xenotransplantation, xenotransplantation product itself, or other relevant tissue, should be evaluated for the presence of infectious agents by appropriate assays and histopathology prior to transplantation, and then archived."

The archives were a long-term archive. It was meant to store specimens for up to 50 years, it would be readily accessible to the PHS, and linked to both source animal and recipient health records.

From the source animal, a number of specimens would be collected, one would be plasma for subsequent serology and viral testing. This was at least one hundred 5-ml aliquots of citrated EDTA plasma.

In addition, there were to be cryopreserved leukocytes; this was to be used for isolation of nucleic acids and proteins. In addition, viable cells should be stored for viral co-culture assays, or other tissue culture assays, and at least 5 aliquots of viable cells, 10 to 7 per vial, would be collected.

In addition, paraffin-embedded formalin-fixed and cryopreserved tissue samples from source animal organs relative to the protocol would be collected. Cryopreserved tissue specimens representative of the major organ systems—for instance, spleen, liver, bone marrow, central nervous system, lung, et cetera—would be collected from the source animal at time of necropsy.

For the recipients, the type and quantity would vary, depending on clinical the procedure and the age of recipient. At selected time points, three to five half-cc aliquots of citrated or EDTA plasma would be collected. In addition, two aliquots of viable cells would be cryopreserved, and specimens from any xenotransplantation product that is later removed.

These specimens would be collected over a period of time, there would be two pretransplantation sets, one month apart, one immediately prior to the transplantation. In addition, there would be sets collected at one and six months post-transplantation, and then annually for the first two years post-transplantation. Thereafter, it would be every five years for the remainder of the recipient's life. More frequent archiving would be indicated depending on the protocol or the patient's course.

At this time I am prepared to say that we now have an archiving place. The decision was made to establish this archive at the Centers for Disease Control in Atlanta, would be part of our existing specimen data bank, which is known as CASPIR, CDC and ATSDR specimen packaging inventory repository system. This would store things cryopreserved things in liquid nitrogen for long-term storage, it would be linked to the National Xenotransplantation Database, which is maintained by FDA, and currently is sponsored by CDC and HRSA.

The information on how specimens could be submitted to this archive, and what configuration the specimens need to be, and how they need to be labeled, this information will be printed out in the near future. If anyone needs further information, they may contact me, and this information should be in your packet. Thank you very much.

DR. GROESCH: Thank you, Tom. Our next speaker with an update, is Dr. Michael Chang from the NIH National Center for Research Resources. And Dr. Chang will be giving us an update on the National Swine Resource and Research Center. We've previously heard about this from Dr. Franziska Grieder, who is here today, and look forward to hearing about the progress that has been made.

DR. CHANG: Good morning. I'd like to update you about the new Swine Resource Center, which is funded by the National Institutes of Health, and to tell you about the center's function, and let you know how the xenotransplantation community, as well as the other biomedical research community, can benefit from this valuable resource.

Now last year at the last SACX meeting Dr. Grieder outlined an ongoing RFA for the establishment of the Resource Center. Now the objectives were to deposit, maintain, preserve and distribute swine models for studies of human diseases. Now we received a number of outstanding applications, and the applications were reviewed, peer reviewed in the summer. From this, an institution, the University of Missouri, was awarded a grant to become the National Swine Resource and Research Center.

The principal investigators are Drs. Lela Riley, John Critser, and Randy Prather. The overall goal of the Resource Center is to provide valuable swine models to investigators, and to shift the burden for maintaining and distributing the unique swine models from individual investigators to a national resource center, and to perform research aimed at improving the swine as an animal model.

Now, the grant was awarded in September of 2003 by the National Center for Research Resources, and cofunded from the National Heart, Lung, and Blood Institute, as well as the National Institute of Allergy and Infectious Diseases.

The Swine Center functions are the importation of swine models, rederivation, cryopreservation, phenotyping, infectious disease screening, genotyping, production, and distribution, as well as research. The first and foremost function is the importation of existing swine models. This is a basic function, and so we are requesting researchers that have developed pig models, especially genetically modified models, to make them available to the new center for curation, production, and distribution to biomedical researchers.

Another important function is rederivation. This is important with respect to pathogens, housing, and production, as well as another service function is the cryopreservation of embryos with spermatozoa and ovarian tissue from each pig line.

The center will also monitor for various pathogens, bacterial, viral, and parasitic use in different approaches; and another important component is the creation of genetically modified swine models, especially for NIH-funded researchers. Now, this will be a service, and one has to apply for this service, and one can do this online, and there is an External Advisory Committee that is set up to review such requests.

Another essential function of the center is the production and distribution of these swine models, and they will provide live animals as well as cryopreserved germ plasm. With respect to live animals, they will provide two to three breeding pairs, and of course the health status and genotype will be confirmed prior to shipment, and this will be at a nominal cost. The resource center, at this point in time, is housed in a temporary facility off the university campus.

In September of 2003, the university was also awarded a construction grant from the Division of Research Infrastructure at NCRR to build a new facility that will house around 175 to 200 pigs, and will provide biosecurity to prevent pathogen entry, as well as it will contain laboratory, as well as surgical space. It is in the design phase right now, and ground-breaking is supposed to take place in the fall of this year, and construction around a year, so the new center will be opening in 2005.

How does one improve the resource? This is done through research, and their research component is to develop improved cryopreservation methods for swine gametes and embryos, to develop improved health and genetically monitoring approaching, and to develop—by improving the efficiencies and creation of genetically modified pigs. They now have a Web site. The Web site information is in your package, and will give you information about the center. It will have the application forms for the submission of pigs, which can be reviewed by an External Advisory Committee, as well as application for the request of pig lines and the creation of genetically modified animals. It will also list the number of lines being developed and available via the Resource Center. Thank you.

DR. GROESCH: Thank you very much. Our final speaker in this section is Dr. Eda Bloom from the Food and Drug Administration. She'll be reporting on a World Health Organization meeting, and some of the outcomes from that, and this was a meeting in Madrid last fall on ethics, access, and safety in tissue and organ donation and transplantation, issues of global concern.

DR. BLOOM: Thank you, it was a real privilege to represent the U.S. along with Dr. Laura St. Martin from HRSA at this WHO consultation that took place last fall. The meeting was entitled Ethics, Access, and Safety in Tissue and Organ Transplantation, Issues of Global Concern, and as you know, it took place in October. The issues of concern that were discussed included allo- and xenotransplantation, and the transplantation of organs, cells, and tissues. I'm going to concentrate on the xenotransplantation aspect of that meeting for this audience.

There were a number of participating countries from all continents, except Antarctica, and in addition to that, there were representatives from not just WHO, but PAHO, which is the Pan-American Health Organization, which I understand is part of WHO. Also the Council of the European Commission were represented, so it was really quite a broad spectrum
of representation at this meeting.

The results were quite remarkable, I think. There was a report by the Secretariat, and the number of the report, which mean more later is EB113 and 14, and that report includes a draft resolution, the resolution was presented at the WHO executive board meeting just last month, and the WHO executive meeting considered the consultation report, and recommended that the 57th World Health Assembly, which will convene in May of this year, adopt the following resolutions regarding xenotransplantation—I don't know how many of you have read WHO resolutions, but they're really very nicely laid out. They start with the rationale for the resolution, and the rationale is usually a list of things that say "Concerned about, cognizant of," and so forth, and in this particular resolution, the rationale for the recommendation portions regarding xenotransplantation were recognizing that living xenogeneic cells, tissues, or organs, and human bodily fluids, cells, tissues, or organs that have had ex vivo contact with these living xenogeneic materials have the potential to be used in human beings when suitable human material is not available. If that sounds a little bit like the U.S. definition of xenotransplantation, it is very much like that. Also mindful of the risk associated with xenotransplantation of the transmission of known, as yet unrecognized xenogeneic infectious agents from animals to human beings, and from xenotransplantation recipients to their contacts, and to the public at large, which basically states the reason for WHO concern.

The executive board urges member states to allow xenotransplantation only when effective national regulatory controls and surveillance mechanisms overseen by national health authorities are in place. It urges member states to cooperate in written formulation and recommendations and guidelines to harmonize global practices, including protective measures to minimize or prevent the potential secondary transmission of any xenogeneic infectious agent that could have infected recipients of xenotransplantations, xenotransplantation products of xenotransplants or contacts of recipients, and especially transmission across national borders. It also urged member states to support international collaboration for prevention and surveillance of infections resulting from xenotransplantation.

Now the resolution, and maybe this is how all WHO resolutions are structured, I'm not sure, are broken up into two parts, one part is urging member states to do something, and the other part is requesting that the WHO Director General do something. And what it requests WHO to do is to provide leadership through the promotion, facilitation of communication and international collaboration among health authorities in member states on issues relating to xenotransplantation, to collect data globally for the evaluation practices in xenotransplantation, and I believe what that means is that WHO is asking the Director General to provide for collection of information so that WHO will know what countries are having ongoing xenotransplantation, and that will make the issue easier to deal with. To provide a response to request from member states, technical support and strengthening capacity and expertise in the field of xenotransplantation, including policy-making and oversight by national regulatory authorities. And so that is saying if member states ask for information, then WHO can provide it, and it can support the collaboration so that countries can then share information.

Now, everything I've said is available at the WHO Web site, which you have in your packets. It is not the easiest to navigate, at least it wasn't for me, so I've actually listed what you do. You go to "Governance," you go to "Executive Board," and so forth, and where the two documents can be found, the two documents being the Support of the Secretariat from the Consultation and the Resolution that will be provided to the World Health Assembly. Thank you.

DR. GROESCH: Thank you, Eda. I am happy to say we are not yet behind schedule, so we have some time if anybody wants to ask questions of any of the speakers that have provided updates today.

DR. VANDERPOOL: Eda is an excellent representative of the U.S. working, and has been with xenotransplantation issues for a very long time. Were there any other members of the SACX committee at that World Health Organization meeting?

DR. BLOOM: There were—it was a very small meeting. There were 30-some people there. Laura and I were both there, both ex officio members, and the other U.S. representatives, and there were four U.S. representatives, which was the most had by any country, was a transplant surgeon, Frank Demonico, who is a former president, I believe, of the Transplantation Society and Nancy Hugh-Schrepp—something—Schrepp-Hughes, I don't remember exactly her name, but she is head of Organs International, which is a humanitarian organization that deals with organ trafficking.

I want to remind you that the meeting was having to do with transplantation in general, and although xenotransplantation in our Venn diagram is a huge circle, it wasn't the major thrust of the meeting, although it was given considerable consideration.

DR. VANDERPOOL: Good. As I question the light of one of our charges being that we would involve ourselves on international policies and development, so it seems to me that kind of meeting is certainly pertinent for some of our—for SACX representation.

MS. SHAPIRO: Eda, I'm just interested in operationally how anything really happens. I mean it all sounded great, but who in this country, for example, will know and do anything about recommendations that may be issued?

DR. BLOOM: As I understand it, WHO documents are recommendations. In this country, we are probably in pretty good shape already being in compliance with what WHO is asking, and are very happy to share as appropriate and if requested our information. I don't know that there are real teeth to make other countries comply. I think today, if he is not here already, there will be a representative from PAHO, which is the Pan-American Health

Organization, and he may have some information on that, but it is my understanding that there is not a method of enforcing compliance.

DR. SALOMON: By the way, Eda, it is Nancy Shepherd Hughes. I know that because she was my daughter's thesis advisor at Berkeley. The question I had, actually, was for Dr. Chang. so this—I mean it is a wonderful resource that is being developed, and I strongly congratulate the group that you have put together there. That is no minor exercise in developing a new resource, and also the support from NCRR and NIH.

The question I had was just operationally, how would you do access, for example, to the small biotech companies that obviously can't afford this sort of infrastructure investment, but would want to move forward in a Phase III trials in xenotransplantation? Are you set up to work with them, and—

DR. CHANG: With respect to them, getting animals from the Resource Center?

DR. SALOMON: Exactly. I mean that is going to be a big issue, right?

DR. CHANG: Yes.

DR. SALOMON: Any given company will give a technology to a certain point through clinical trials, and at the point they want to do a clinical trial, the key would be to have access to designated pathogen-free animals, and to have this kind of tremendous scientific background to make sure that the animals are screened properly, et cetera?

DR. CHANG: Well, right now we are—the center just got started, and so the principal investigators are trying to talk to people that have developed these models in order to have them donate them there. And of course there will be MTA issues, and hopefully it will work out, in the future with respect to this. We haven't really come up with a plan of involving, as yet these biotech companies.

DR. SALOMON: My comment would only be that you should work on a plan like that.

DR. CHANG: Yes.

DR. SALOMON: It is critical from all the different scientific points of view to develop these models and advance research in that area. It is clear you are set up well to do that. But the other need, I think, will be in translation of research going on in the different biotech companies, and I would say that it is also true that some of these advances toward preclinical and then in the clinical phase have come from other countries as well, and you are going to have a unique resource. If every small company had to come up with, you know, a hundred million dollars, or whatever, that is going to eventually go into your facility, it would delay the field, so it would really be great if you could develop reasonable ways for access.

DR. CHANG: I'll share that with the University of Missouri, and update you next time on such plans.

MR. BERGER: Eda, I had noted in Tom's talk that the archives would be linked to the National Xenotransplantation Database, so the question is how about an update?

DR. BLOOM: The National Xenotransplantation Database is in a format to be used. What we have to do now is publish a guidance document so people know how to use it. That guidance document is written, and it is undergoing agency clearance. We developed a number of forums that we hope will facilitate submission of information to the database. Those need approval, as understandably by FDA, but they need to go through OMB approval. The approval process is going to be that of the usual guidance, which means publication, comment, and then again publication in final form, and part of that comment will be on the paperwork aspects, or the performance aspect of it. So as far as actual submission of data to it, that may be a while. I don't want to give a talk about that.

MR. BERGER: Are you planning to go back, in terms of trials that have already been done, in order to put people from prior years on this database?

DR. BLOOM: As much as possible, yes.

DR. VANDERPOOL: I want to join the voice of Dr. Salomon to congratulate Dr. Chang and all those who made this national center possible. It is very exciting, and we have been concerned about the quality of control, and duplication, extra funding duplication, and all kinds of things. But this is a very exciting development, in terms of the future of access to swine who have been reared in closed colonies, and so on. Very welcome, very exciting piece of work that you have done.

DR. ALLAN: I have a question, just as a follow-up, I may have missed this, but specifically, in terms of how they will provide pigs for investigators, do you envision that you'd be providing individual pigs for transplantation? Because it looked like you were talking about breeding pairs, so would that suggest, then, that an investigator would have to have a facility in which to breed pigs, or would you be providing individual animals?

DR. CHANG: Well, it depends on need. Of course, as I said, they were just set up. Ideally, to provide breeding pairs, and, yes, they would have to have a facility. But there also, if the request is to have pigs for use within a few months, I'm sure the center could provide such an animal. Also they're building a new facility which could also increase production for investigators in that respect.

DR. GROESCH: Any other questions or comments on our first presentations?

Okay, I think we can move on to our—the next part of our meeting, which is discussion of one of the committee reports. This one is the Draft Report on Informed Consent Issues in Clinical Research Involving Xenotransplantation. And we realize that members of the audience haven't had the opportunity to look at the draft report in advance, and so we are going to provide an overview, and that will be done by Robyn Shapiro, who is a member of the committee, and also was one of the co-chairs along with Dr. Vanderpool of the working group on informed consent.

Agenda Item: Overview of Draft Report on Informed Consent in Clinical Research Involving Xenotransplantation

MS. SHAPIRO: Thank you. It was—I just want to say that it was a real pleasure to work with our group on this report, and we're anxious to hear comments from you.

We started out with what were we going to talk about? And obviously there are many ethical issues, social, economic, medical issues associated with xenotransplantation, and we decided to focus in on informed consent for a couple of reasons: One, because we already are seeing this, we'll hear about later today, and as we have heard about, limited clinical trials involving xenotransplantation of tissues and cells; and, two, because we feel that it is inevitable that soon we will again see clinical trials involving solid organs, so that this issue is of immediate and urgent importance.

The goals really are twofold, and when you have a chance to read the report, you'll see that first we wanted to give a general description about important issues in informed consent that surround any complex research endeavor, and then we wanted to focus in on some of the specifically challenging issues that relate to xenotransplantation. We'll get to those very briefly soon.

So we started with the general discussion of informed consent, and as Dr. Vanderpool will do as the good biologist that he is, first and foremost we had to look at the ethical foundations and functions of informed consent, which boil down to respect for persons, and autonomous choice. And these are reflected in a number of medical ethics codes, in law, in the literature. Then we go on to talk about the components of informed consent, which again boil down to include disclosure of relevant information, comprehension, on the part of the prospective subject of that information, and voluntariness on the part of the subject in agreeing to participate.

We then looked at the process of informed consent, focusing on issues such as the content of what is to be disclosed, the individuals involved in that disclosure, the setting, the format, the pacing of that discussion. And finally,well, actually, I am going to plug here, in the process here, we talk a bit, one unique aspect, perhaps, is the notion of the consent team, which we will include in our recommendations at the end.

A team, given the medical complexity of what we're talking about here, the lifetime commitment of follow-up, which again I'll get back to in a moment, the potential public health, psychological, psychosocial financial issues, we recommend a consent team to include the principal investigator, an individual knowledgeable about post-transplant care and long-term responsibilities of recipients, and an individual who has expertise in psychological, financial, psychosocial implications, with others available to talk to the individual in the informed consent process. Then in our report, we go to informed consent forms, highlighting throughout that the form is only one part of the process, not a legal document to protect against legal liability, primarily, but one part of the informed consent process, and we include a model informed consent form, which we think provides a clear, well-formatted, comprehensive, understandable consent form for xenotransplant protocols, generically speaking, that is.

Then the report gets to the special issues raised by xenotransplantation research, with all of the foregoing as background, and there are several: Public safety measures, due, of course, to the risk of spreading new infectious disease, some of the subtopics in that general category include the necessity for long-term surveillance of xenotransplant recipients, the routine physicals, the lab tests, and the archiving, and future testing specimens, the autopsy, isolation, or the
possible need for isolation, if there is an imminent risk of casual transmission of infectious disease presented through a xenotransplant procedure, and the possibility of quarantine, if again there is infectious disease risks that poses a serious and immediate health threat, and non-compliance on the part of the recipient to comply with public health protection measures. So we talk about those issues.

We also talk about some unanswered questions at the moment, that is, what do you do when you have an asymptomatic xenotransplant recipient who simply refuses, or fails to comply with the surveillance requirements or instructions? And we note there that under the current law that we have in the states, because public health laws are mostly state by state, there really is no mandatory periodic monitoring measures that could be called on.

We then go to issues involving third parties, which are of course particularly challenging, and the third parties fall into a number of categories. First of all, we have intimate contacts, and the
question that we looked at was, well, since they are at some risk of also acquiring infectious disease, do we have to get their consent for the xenotransplant procedure to be performed on their contact? And there is no way to do that. We don't see that happening in clinical research accommodated by law, and it is also practically difficult. Intimate contacts, of course, change over time. So do you try to get the consent of the current intimate contact, or somebody who you think will be an intimate contact in the future of the xenotransplant recipient? It also involves the disclosure of confidential information about the recipient, so the conclusion that we came to in our recommendation is that the recipient needs to be informed of his or her responsibility to educate both current and future intimate contacts about the possibility of infection, with help in doing this disclosure, if wanted by the consent team.

Health care professionals is another group of third parties about whom we had concern and focused, concluding that the informed consent process should include a component that advises recipients of xenotransplant products of, again, their responsibility to advise both their current and their future health care providers of the fact that they are a recipient. The providers themselves involved in the procedure should be informed about the risk that they face, not just to minimize that risk, the need to report significant unexplained illnesses, and the sponsor, or the center where the procedure is performed, should have plans for monitoring and for post-exposure evaluation and management.

The third group of third parties is the community, and this posed additional challenges for us. do we need to, for example, get the consent of the community, because there is a possibility that all of us would also face some risk of disease? Well, how do you do that, and what is the community in this day and age when people move and travel?

So we concluded that perhaps a better paradigm is community consultation, as opposed to community consent, and that this committee, or one similarly situated, could be very important in helping to ensure that there is public education and discourse about not only the public health, but the medical, the ethical and the social issues that are associated with xenotransplantation through meetings like this, through the development and distribution of information and resources, through developing collaborative relationships with counterparts in other countries, which Eda talked to us about, and is helping with, and through other activities.

Then we turn to issues involving informed consent when the potential recipient of a xenotransplant product would be either an incapacitated adult or a child. We started off

this section by talking about what the definition of decision-making incapacity is for these purposes, and what the regulations currently offer, which is not much guidance in any clinical research, for involving, or enrolling incapacitated individuals, and then we did look specifically at xenotransplant research involving incapacitated adults. And our recommendation was to limit those persons' enrollment to those in whom mental capacity is likely to be restored by the xenotransplant procedure, and also to require that there be evidence that this individual would have wanted to participate, or that participation would further his or her own best interests, and that we have some evidence that this individual is responsible, so that we can anticipate that when hopefully capacity would return, they'd be likely to adhere to the lifelong surveillance requirements. And finally that there are some plans for assistance in meeting the long-term surveillance needs, if those would be required. Those are the conditions under which we thought that enrollment of incapacitated people, adults in xenotransplant research protocols, might be appropriate.

And then we went to children, also difficult, in light of ethics and federal regulations, which for the most part say that parents or guardians should not enroll children, unless there is not significant possibility that the child would be helped by enrollment, so our recommendation was with respect to children, that as a general matter, they should not participate, and that the exceptions might be only those cases where the possibility of benefit to the child would be high, given the alternatives, and that this would be determined on a case-by-case basis.

And we end with a series of recommendations, and I don't know if we have time to run through these? We do have time to run through these? Good. The first is that the informed consent process should ensure complete disclosure of information, comprehension on the part of the prospective recipient, and voluntariness in the decision-making process. Again, those go back to not just xenotransplantation, but the literature that talks about what informed consent should be ideally in all cases in research.

Second recommendation is that the goals of the informed consent process should be facilitated by involving a research team, as I described it, by holding a series of face-to-face discussions about the protocol in proper settings, this is, of course, if time allows, and by using an informed consent form that is understandable, that includes information that is required by regulations that are out there, as well as those that have been recommended by some of the federal agencies that have weighed in on this.

Third, the informed consent process should include an understanding and an agreement on the part of the recipient to comply with the public safety measures that I talked about before, in terms of the lifelong monitoring and so forth, and to educate his or her current and future contacts about these risks and these requirements.

Fourth, that public health organizations, or public health departments should maintain good communication with physicians, who are likely to serve as the first line of defense against any new disease that might pop up in xenotransplant recipients.

Fifth, that our legislatures, state by state, evaluate the effectiveness of their current public health laws to address the situation that I briefly touched on, and that is the asymptomatic recipient who simply fails to comply with the surveillance instructions. For the most part, our public health laws are very old, and they do not fit well with this possibility.

Next, that health care workers who are involved in xenotransplant procedures be adequately informed of the risks involved, as well as monitoring plans well in advance of their participation in performing the procedure.

And, next, that the sponsor, or the institution where the xenotransplant procedure is performed, should have monitoring, as well as post-exposure evaluation and management plans.

Next, that this committee, or one like it, should ensure ongoing education and discourse in the lay community about, again, the public health, the social, the medical, and the ethical issues that surround this kind of research.

Next, that the enrollment of incapacitated adults be limited, again, to the situation where, if likely that mental capacity is going to be restored by the procedure, the legally authorized surrogate decision-maker who is going to enroll this person determines that the enrollment accords with the individual's likely preferences, or, if known, their best interests. That the legally authorized surrogate decision-maker who is going to enroll the incapacitated adult

represents that this individual is generally responsible, likely to adhere to follow-up responsibilities, and that there are plans for assistance with a lifelong follow-up, if it is needed.

And, finally, that children should not participate in xenotransplant research at this time, except in special circumstances where the possibility of benefit to them, ah, is high, given the alternatives. Questions?

DR. VANDERPOOL: Thanks so much, Robyn, for that overview. At this point in our schedule, we have that public comments should follow the committee's thorough discussion of this document. I want to, as chair and facilitator of this discussion, to propose that the public comments, if you in the public wish to make comments about what you've heard about this report, feel free to do that at this time so that we can, as a full committee, can take your concerns into consideration as we proceed.

So if you—If you, a member of the public or media have a question or comment, please feel free to make that at the present time.

Agenda Item: Plenary Discussion and Public Comment on Draft Report on Informed Consent in Clinical Research Involving Xenotransplantation

DR. COOPER: Yes, I have a question. With regard to children, it is unlikely they are going to be offered xenotransplantation unless the benefits are going to be very high, and therefore they will be included, presumably. But when we were writing the report for the international study of heart and lung transplantation, it was made clear to me by pediatricians that parents were very upset if children would be excluded. So I think that requires perhaps a little bit more thought, or a little bit more wording, because to advise that children would normally be excluded, I think, is probably contrary to what parents and pediatricians would advise.

DR. VANDERPOOL: Our intent, David, was not to exclude them, but to make a strong statement that they would generally not be included, unless the conditions were to warrant it, which it seems to me would be in keeping with your suggestion, that if the benefits seem to be the very best available, that our report is saying that we could be open to enrolling children, of course we have in the background the Baby Faye case, which created enormous controversy, so one of our concerns is to issue a warning, but not close the door. Dan Salomon.

DR. SALOMON: This issue with children, and in research, is of course been that is not new, and it is not easy to resolve. I remember coming to the NIH, almost 15 years ago with a group, that was proposing to use cyclosporine to treat children in the honeymoon period right after the development of insulin-dependent diabetes, and we met with a panel of pediatricians and endocrinologists at the time who were extraordinarily concerned about the risks of cyclosporine, and you can guess that my job was there to talk about cyclosporine immunosuppression, and try and assuage some of the fears of that, though there clearly are issues with using immunosuppression in children. What is really, I think, a crux of the position here is that nobody should be denying children participation in cutting-edge clinical trials where there is significant benefit.

On the other hand, parents way too often take a position of extraordinary optimism on new interventions that are not warranted, if you take a good, hard and cold look at the realities and the risks, so I think that that is where the balance has to be.

DR. VANDERPOOL: Comments from the public? Identify yourself first, please, and then ask your question or make your comment.

MS. STIFANO: I'm Toni Stifano, I'm with the FDA, and I have a question. Again, you know, thinking of the issue of children and thinking the issue of public awareness of the complexities of xenotransplantation, when—and not having read your document, when you talk about urging legislators to perhaps go back and look at the law, are one of the things that you are considering that the concept of "informed consent" be broadened to include family members involved? And are you planning to—on having, in terms of clarifying—looking at the state of informed consents as a whole now, it is pretty abysmal, in terms of comprehension and volunteerism. In other words, you know to say many of them, and I just looked at one that said, you can do this, or you can go for another treatment, but you'll have to pay for it. And knowing that the cost of this alternative treatment that is already on the market, acceptable treatment is very expensive. So how are you, when you are talking about seeking comment, asking for

specifics in terms of content? Are you asking for—I'm trying to understand what it is that the function of this document is? Is it to get everyone to think out of the box, and think maybe it is not an informed consent for the family members, but it is an educational piece that everyone signs, agrees, and so on, and they know things to be mindful of.

MS. SHAPIRO: Maybe I can respond to some. I think you brought up a lot of issues. We are not, when we are urging legislatures to look at their state laws, the focus of that comment was not to change the law about informed consent. It was to change the law about public health measures that can be utilized when you have an asymptomatic individual who is not—I mean I think that your point about informed consent not working is very well taken. I don't think the law is going to fix that, but I think it is very well taken.

And with respect to the kids, I think another complicating issue about the enrollment of children is not just the usual risk/benefit problem, but also the fact that the commitment to the lifelong monitoring is involved, and I think that that makes the issue with respect to consent on behalf of children all the more complex.

DR. VANDERPOOL: Comment?

MR. COOK: My name is David Cook, and I am from the U.K. Xeno, and we have been through a similar exercise. Two comments, one, I'm surprised at the strong emphasis on the individual, particularly the first recipients are not likely to be well, and whether or not they have the capacity in order to inform and to educate close contacts. U.K. Xeno, when looking to this particular issue, decided that we would try to get consent from intimate contacts, recognizing that in the end it meant that some patients would not be treated because family members would not be compliant.

DR. VANDERPOOL: We still have to make a distinction between informed consent before actually receiving the organ or tissues involved, and being educated about the sequelae of dealing with being with the recipient. In a sense, the education we are urging is implied consent, but there is nothing in the law that would say that they would need a consent form, since they wouldn't be undergoing the procedure themselves. We also accent in the report that the individual who is to be the recipient is by no means the only one to do the educating. As the federal guidelines point out, there needs to be, and will be, according to this recommendation, a significant amount of assistance in that education. We also urge that at times if the prospective subject of the research agrees, because otherwise privacy is broken, that the consenting team would meet separately with the intimate contacts and talk with them. So we definitely recognize the role of the intimate contacts. It would be a counseling matter if someone said, "Well, I don't want my spouse to receive this organ," but he persists or something like that. I think that is—I think that is putting—this document would be a hundred pages long, instead of 30, to make for those exceptions.

But, if such cases arise, one of the purposes for having a, quote, consenting team, end quote, which would involve the primary investigator, someone well associated with the social and psychological and public health issues, and then a personal counselor of some kind would certainly field those issues with the significant others of the prospective recipients.
Other comments from the group on that? Bill.

DR. SCHECKLER: Yeah, I have sort of three general comments, one on public health laws, the second on the thrust of the whole report, and the third probably my controversial one, on infectious disease issues.

First of all, on public health laws, there is a process now completed of a model public health law both for general public health for quarantine, infectious disease agents, about bioterrorism, and all related new agents that has just been promulgated, and is available, and having chaired the committee that rewrote the Wisconsin Public Health Statutes in 1991, I have been aware of this process, so that most of the state laws since 10/4/01, since the anthrax outbreak, have been looked at, and are in the process of being updated. So, Robyn, I'll try to get you a copy of the new laws, because that is a major improvement that has happened just in the last two years.

Second, the comment on the general, I particularly like the notion of the team, of the fact that the form should be written in understandable language, and this is coming from having served on our University of Wisconsin Medical School IRB for a number of years, and having formats other than just the consent form. People tend to obsess over the written form of the chart, and if that is the answer to consent, as you very nicely structure in this report, I think

those processes are all very important and very useful, not only for xeno, but probably for anything that is new and different.

The third thing is the infectious disease issues. I think that the goal, both in the science report, and in this report, is the fiction of zero risk from infections, and I think that is a fiction. I think that we're building a house of hypotheticals, and that you are struggling with our house of hypotheticals. By that I mean I think there is no, and I have waded through the 20 pounds of information that we were given at the beginning of this process three years ago trying, particularly with my background on infectious disease and epidemiology, to focus on what is—what do we know what has happened, and what is biologically plausible? And I would suggest to you that even the World Health Organization comments that Eda made, that we are just wringing our hands for no particular good scientific reason. There is zero threat to the general public from xeno. There is zero threat to casual contacts from xeno, from anything that we know about, any of the viruses, retroviruses or other things, there is probably almost no problem with family contacts, there might be some problems with retroviruses, maybe in intimate contacts. But realize the HIV epidemic got started by perfectly healthy people. Xenotransplant recipients aren't perfectly healthy people. They have end organ failure of something, and they aren't allowed—they aren't likely to be jetting around the world, like some of the stewards on planes spreading their AIDS all over the world.

So I think that we know about porcine endogenous retrovirus. We now know more than we ever thought we should know about that, and we are taking that in some of our experience with retroviruses, and we are building this fiction of zero risk from infection, and I think that has just been a huge barrier. The barrier comes in lifelong follow-up, the barrier comes in risk to everybody in the world if we get it wrong, the barrier comes from lack of understanding, and people are using, even the essay in here by Fishman, misunderstanding of how SARS is spread, misunderstanding of the genesis of respiratory spread illnesses, droplet spread illnesses, and other kinds of illnesses that do put people at risk, and just obsessing over it. And I think this fundamental hypothesis that both the science and the ethical committees have been working under is a false hypothesis, that zero risk isn't possible, and that we are putting up a huge barrier to moving forward in this field, and I think we ought to recognize that.

DR. VANDERPOOL: Okay. Do we have other comments from the public on the issue of this report? Dr. Valdes, do you have—

DR. VALDES: I would like to remind you how we can define children. Children is one year, two years, three years, five years, so I would like to remind you what is the definition of "children"? One year, six years? I have a daughter which is 26 years, and I used to call my child.

MS. SHAPIRO: In the report, we defer to state law, which varies a little bit, but in all states, there is a statute that says you are an adult at, and Wisconsin, for example, is 18.

DR. VANDERPOOL: Which would mean that someone is a child any time they are under that age. So I would remind our committee we probably need to change one little phrase in the report about informed consent for children in the U.S. It is not informed consent, it is parental permission and childhood assent. We do mention that later on, but informed consent for a child is a fiction. It is parental permission, which is equivalent to the information on the informed consent form, and childhood assent, which is the entire research project explained, as well as possible to the child, depending on what the child's age is, so you have a significant difference in the assent form for a child who is six, versus a child who is 12, versus an adolescent who is 17.

One comment about Dr. Scheckler's address. We'll certainly get to this issue in the state of the science report. As far as our report on informed consent is concerned, I press you to find anything about our saying that risk has anything to do with zero. Our concern here is that the risks are very clearly forthrightly and honestly described. That is the way we press all the time, and with that regard, the risk may be seen as lesser now, and far greater and weak, whatever that risk is, however it is related to the actual procedure, has to, according to this report, be clearly defined, clearly stated in commonsensical terms for the prospective subject.

All right, I welcome, of course, I think we all welcome, all the subcommittee welcomes your comments about what we have done with the model consent form. We spent a lot of time on that form. I think it is a wonderful form and discussion for complex research in any area, not just xenotransplantation. Dr. Collins and several of us spent a lot of time talking about what all should be in this form. Everything there is informed by the guidance documents, by

ethics literature, by IRB literature, by OHRP literature. And so we wanted a full grid of issues, but we also wanted to use commonsensical language, clear and large print, so it would be comprehensible, and so on. So I welcome your comments there. I think these are useful and applicable to IRBs across the U.S. Other comments? Yes, Thomas.

DR. SPIRA: One of the major issues in the document is to ensure comprehension in the informed consent process, and one of the things that some studies have done, where they are either at grave minimal risk, highly complex issues involved, is to have some measure of comprehension. The study that comes to my mind is that of the HIV vaccine, where there were a lot of implications to the subject, and also the concerns about changes in behavior based on being in the study. And in that study, there was a test the person had to take. If they didn't pass the test the first time, they were re-educated, re-informed in the consent process, and re-tested, and if they didn't pass the second time, then as I understand it, they could not be in the study. Have you considered some process like this in measuring comprehension, rather than just going through the process and just assuming comprehension?

DR. VANDERPOOL: I think one of the innovative sides of this paper is the excellent discussion on process. Kathy Crone, a number of others on the committee, spent a great deal of effort and time on the process issues. And it is—and it includes everything from discussions, to questions and answers, to feedback questions, can you tell us what you just said, to.
Multimedia possibilities. So I think the thrust of the report is to see that comprehension does occur.

As you surely realize, for very sick persons, or for persons in general, one can argue that it is paternalistic to give them a test that they have to pass before they can enroll in a protocol. And to force comprehension of every detail that we take to be important is to assume a paternalistic attitude as to what is important for us, and not necessarily recognize what is important for that particular subject.

So the question of fully comprehending according to an examination what the protocol is about, is open. I definitely can understand why we need better comprehension in the studies very often. We do need better comprehension, but I think it is in the spirit of this report that the comprehension should be thorough, and we outline what procedures need to be done in order for that to occur.

Other general comments? I want to go through the very specific comments that we have certainly before we end, first from the public, and then from Dr. Kalin.

DR. SYKES: I have a comment on the issue of the mentally incapacitated individual. I would think that—I mean obviously there are many cases of acute pulmonary hepatic failure where you really couldn't preemptively obtain consent, but I think it might be advisable to include a recommendation that whenever possible, the consent be obtained preemptively, in anticipation that the person might deteriorate, somebody who is on a waiting list for a liver, and ultimately can become incapacitated, they could be—they could have this discussion before that occurs, because I think that, having a surrogate give consent and try to provide evidence of the person's responsibility and wishes is a very difficult thing. Obviously the surrogate is going to be somebody with a vested interest in seeing the person's life saved. And so I think that situation should be avoided whenever possible.

And also I wonder if we could be, perhaps, more specific in the level of evidence that would be required. I mean I think that one could say that somebody who pays their taxes and their bills is responsible. But that, to me, would be a fairly low level of evidence of that person's sense of social responsibility. I think we should try to make some effort to define what level would be required.

DR. VANDERPOOL: Those are certainly welcome, and can be easily put within that section, Dr. Sykes. Comment.

MR. VASCONCELLOS: Al Vasconcellos, LCT BioPharma. I would ask the committee if a mechanism might not be included in reports of this nature that would dynamically address and evaluate the data gathered over time, and then adjust and correct, if necessary, the guidelines and procedures associated with things like informed consent and many of the other components that you guys will be deciding in the near future.

DR. VANDERPOOL: That is an excellent comment. If we don't have a statement to that effect, we need to put that in there, namely that the informed consent that first appears on this form is not necessarily the—is not the only information that needs to be conveyed. That this information will vary over time and will need to be adjusted, additions added and so on, that is very helpful, thank you for that comment.

MR. VASCONCELLOS: If I might add also if Dr. Scheckler is correct, and time proves that xenotransplantation is a great boon without secondary problems, the adjustments and making the introduction of new xenotransplant products easier, simpler, more effective.

DR. VANDERPOOL: Okay, Sharon, Dr. Kiely.

DR. KIELY: Thank you, Dr. Vanderpool. I think we've gone a long way in this process, as you know, to making the informed consent process more applicable to xenotransplantation. But given the space of time we've had as a group, I read through this a couple times, and there is something that jumped out at me, and I don't think we need to do very much to address this issue if the committee feels they would like to, but I think in dealing with respect for persons, and, you know, disclosure of information, we've done along job in telling them what we think the risks might be and their responsibilities, but I think we've sort of dropped the ball on what their rights are, the individual's rights. And I point out like three examples of this. On page 12, we talk about the placebo control group, and certainly the risks and benefits of being potentially in the placebo of such a study would be explained, but are we to say that that group needs to have lifelong surveillance? And I just put that out to the group. And then on page 14, we talked about the participants being informed, and following up, and seeing their physician, but we don't really address the fact that information will be given to the individual, specific information would be given to the individual at the follow-up, and based on their testing that they could use in their personal lives or other.

And then another example on page 16, we talk about that they could lose their job, or lose their health insurance, and we really don't talk about the recourses that are provided by law for the loss of that confidentiality, so the patients' rights, I think, in a couple of examples were, you know, examples where we talked about patients' rights were in alternative treatment would be available to them, and non-discrimination certainly. But I think we could certainly do well to look at the document in that light. And where we're also asking something of the individual, we need to ask again something of the researchers, and others to provide for the patient.

DR. VANDERPOOL: Those are welcome comments. By the way, if you have specific comments, editorial comments, or brief comments to make, by all means feel free to make them on your copies for the committee, and ex officio members, and give them to Dr. Groesch so that we can work these into what we hope to be the final draft. Okay, do we have—yes, Dr. Drew, we are glad to have you as a representative from the OHRP.

MR. DREW: Glad to be here and joining the party late, and have some editorial comments to offer on the report that I'll provide to Mary. A couple of the points in general would be regulatory language to describe the person involved in the study, it is the "subject" as opposed to "participant." I know in back they referred to "participant/subject," but the regulations we live under refer to "subject." I think it is important in a number of places in the report to differentiate between the prospective subject, and the subject who has signed the form and enrolled and participated in the study. And also that benefits to be referred to as "potential benefits" and that if you knew there was a benefit, you should be doing treatment instead of doing a research study.

One point on involvement of children. The requirement for research to be a direct benefit to the child is not absolute, that certainly for—that would be under Section 405, or above "Minimal Risk Research," but under 407, with secretarial consultation and review, there could be research that would not provide direct benefit, but would provide important knowledge for the understanding of a condition so that the bar is not absolute in that case.

DR. VANDERPOOL: Thank you for all those valuable comments, and we certainly will editorialize according to those ideas.

I think under "children," we view this as under the section on benefit, and that we leave precious little room for researchers to go to the secretary with non-beneficial protocol for children. But all your other comments are right to the point about the nature of language we use. I probably have to plead a degree of guilt in putting the "participant"

language in there a time or two, in part because I appeared before the National Bioethics Advisory Commission, and they kept shoving "participants" at me, and I kept putting that "they should be called subjects in my footnotes," and so we use it circumspectly a time or two, but certainly "subjects" is the form of the federal regulations.

Your other points about the way the federal regulations are worded are very well taken. Thank you for those comments.

MR. DREW: Just my personal preferences are for "persons studied" or "people studied," but I haven't had many buyers for that yet.

DR. VANDERPOOL: Good. Well, I think "subjects" is really quite good, because I think the NBAC was reacting to the notion that people are subject to the researcher, which is not the way "subjects" appear. "Subjects" are those who appear as models for artists, those are the subjects. And so "subjects" can have some very positive connotations, and not just the negative, subservient connotation that most of the community members NBAC had in mind. Yes, another public comment.

MR. LUCEY: Charles Lucey, Food and Drug Administration. And one of the comments I'd like to ask the committee to perhaps consider, or speak to, is that this should be a two-way street, that it is not consent, this really, in my mind, should be more of a contractual relationship, and some of the things that are brought out at the NIH Ethics Course, for instance, is should there be a responsibility of the investigator to offer medical care to the recipients for complications or future problems that may result. Many of these people have no insurance, for instance. At NIH a lot of times they could get this medical care because they have the federal health backup, but a lot of investigators out in the rest of the country do not have a section addressing what their responsibilities are to the subjects of interest, what medical care they may offer, things like that.

And there is also a concern that some of these subjects get lost at follow-up, and what is the responsibility of the investigator to go out and find these patients, and make sure they understand what is going on, what their test results are, and what the future consequences might be? It is just sometimes very convenient to say "This person was lost to follow-up," and what happens to that person in the long run? As you point out, what happens to society in the long run? So I think it would also be helpful for the committee, if you are going to ask states to consider new public health language in their laws, to put forth a model suggestion, as the other speaker suggested. There is model legislation being proposed by the CDC and other public health people. I think a specific suggestion how the language should be worded, so there could be a uniform law across the United States, would be very helpful. Thank you.

MS. SHAPIRO: I think those comments are excellent, and we talked about some of it. The problem is at the moment there is really no teeth to do much of what you would like for investigators and perhaps sponsors to do. We would love it if there would be a contractual obligation for the provision of care of something—at no cost to the subject if something happens. But we couldn't find any mechanism with which to impose that. And, in fact, we were talking about the possibility that the sponsor would go out of business, and what would happen then to the individual who had been the subject of a research protocol involving the receipt of a xenotransplant product? And there was really nothing that we could come up with that would force any company that had no money to continue to stay in business to be able to provide that to the recipient. So, we struggled with it. I'm just not sure that we have any good answers.

DR. VANDERPOOL: Okay, final public comment. Yes.

MS. STIFANO: Toni Stifano again, FDA. I'd like to build on a—two comments that were made with regard to comprehension, one of which is to have follow-up, but in the context of rather than testing, maybe pretest, not necessarily in the recipients, but pretest, because in looking at some of them, I started to flip through this, in looking at some of the suggested language, it is not at the sixth or eighth grade level. And this is something—it is woefully true, but the reading levels now, we understand, are somewhere between sixth and eighth grade level. I had the opportunity of taking a course, a two-day course that had to do with comprehension, and we did a lot of work with informed consents, and it really was surprising to hear parents of children who are not able to understand simple concepts when they were asked to repeat back, what do you think, what does this mean to you? And they were not able to do it, and to maybe all of us in this room, it seems very simple, very basic, but that is not necessarily the case

when it gets in the hands of the public, so perhaps the thing to do is to do a test of sorts on comprehension to a public prior to coming up with something that might be comprehensible to the individual, especially in a stressful situation.

DR. VANDERPOOL: Thank you. Yes, John.

DR. ALLAN: Just a quick response to Bill's comment earlier, because people may not be here this afternoon, or when we discuss the science, and I just wanted to make the comment that obviously there is no—you are not going to get a zero risk. That is why we're wringing our hands, because you can't know for sure whether or not you are going to introduce an infectious disease through xenotransplantation. To suggest there is no risk, I don't think anybody would suggest there is no risk. The reason we do wring our hands, we've had meetings for the last, what is it, eight, nine, years talking about infectious disease risks, so I think, I mean this is an area that is a major concern, and that is one of the major charges of this committee, is to discuss and deal with infectious disease risks. So I don't think you are saying that you believe there is no risk, because there is much in the literature that suggests there are virus infections, and there are unquantifiable risks involved in animal-to-human transmission. So I am not really sure the edge to your comment earlier.

DR. VANDERPOOL: Bill.

DR. SCHECKLER: Well, let me try to clarify a little for you, John. Specifically, what I would do with this part of the document is I would delete the third sentence of the first paragraph, where it starts, "It is, however, accompanied by the unquantifiable public health risk," and I would be draconian and delete pages 20 through 23, and leave all of that to the Science Report. You talk about quarantine and lifelong surveillance, public health laws, which I've already touched on, which by the way, the new public health statutes do cover things that would be even theoretically conceived as a problem here. And issues involving third parties, intimate contacts, health care professionals, community consent, that is all predicated on the notion of spread of some unknown agent. What I was saying is it is impossible to get to a zero risk of infection, because that is not biologically plausible. In the city of Pittsburgh, they had a process to say: We are going to get to zero nosocomial infections in all our patients at all times. That is not plausible. That is not biologically plausible. There will be some unknowns. There will always be unknowns, and what I am saying is that the risk to the general public, the risks to casual contacts, and probably the risks to family contacts, and in most circumstances risks to even intimate contacts is as close to zero as we are allowed to get, and I realize people who have been discussing for eight years the hypotheticals on the theoretical on risk for xenotransplantation. The kinds of things that we are talking about in terms of the porcine endo—the PERVS, and the retroviruses, those are the kinds of risks I'm surprised somebody hasn't brought up prion so far. That is a theoretical risk, and you can set up the hypotheticals and the theoreticals forever and never come to any conclusion, and you can set up the barriers of lifelong quarantine, and lifelong maintaining tissue specimens and so forth. I don't have anything against the maintaining the tissue specimens, but the barrier to any kind of informed consent when you say you are going to have to be followed up for the rest of your life, your family has to be concerned about this, the general public has to be concerned about this, with the 24/7 news that we have right now where people obsess over everything, the influenza epidemic this year is a good example. It probably wasn't any more severe than any other influenza epidemic, even in kids, but you'd never know that because we emphasized deaths in children till, you know, every newspaper had an article a day. It is like their editors required them to have an article a day on something horrible, and we just never particularly looked at it before. So the 24/7 thing is going to sink all of this.

And I think—so I am a skeptic, and one thing that I would do that I think we can leave to the science document is all of this, what I call the house of hypotheticals, infectious disease risk, and it isn't necessary, other than to refer to that, to inform the informed consent.

I would also say, as far as my understanding, and you can correct me if I'm wrong, Harold, that the adult informed consent form, this is not the wording, this is not the sixth-grade education wording, this is for the investigator to use as the format to put together a consent form, and I agree with you, this is a very useful part of the process, as is the other things that you have included in here. That is what makes this I think extremely useful.

DR. VANDERPOOL: Thank you, Bill. One of our responsibilities in this document was to put in the document that which is required by the Public Health Service, so that the issues about lifelong monitoring, and all those, are

directly related to what now is required by the Public Health Service, modifications of these in light of possibly, lesser worries about the risk could be forthcoming. But at this point, we are talking to the research community, which has to perform under the guidelines at the federal level, and I think that is one of the reasons why a lot of these things are there, because this document reflects, the federal regulations and compliance with federal regulations and guidance documents.

Could we now move to looking at some of the specifics that were talked about on the page and a third or so of comments, and see if members of the committee have any response to these, and then if you have, if the committee members or ex officio members have other concerns, feel free to raise these as we go along.

The first on page 8, lines 16 and 17, says "These particular guidelines," it's talking about the guidelines from the Food and Drug Administration, the Public Health Service, et cetera, "address subjects' consent to inform their future contacts of their potential risk of infectious—infections from source animals, consent for indefinitely deferring donation of blood and other body parts, and other issues that are dealt with in the informed consent outline provided in this document." And the FDA reviewer says, "These particular guidances, this is confusing."

Do you mean the guidance to suggest that the ICD, Informed Consent Document, include a statement such as "Your spouse will be informed he/she, will be at risk of contracting the disease, or you will not be able to donate blood or organs in the future?" Comments about that?
My quick response is yes, that is what the guidance document is saying, we need to have something to that effect there, you won't be able to donate organs in the future, and you will need to tell your intimate contacts, including your spouse, that you run the risk of whatever risk we can accurately assess for contracting some infection. Other comments? Okay, page 11.

DR. COOPER: Excuse me. I am confused.

DR. VANDERPOOL: Yes.

DR. COOPER: I think it is confusing. Does it mean that the contact is not going to be able to—is going to have to indefinitely defer donation of blood, or is it that the subject will? It is not clear from that statement.

DR. VANDERPOOL: It is the subject, yes.

DR. COOPER: It could be they're informing future contacts that they are going to have to consent to deferring donation, the way it is written, I think that is probably confusing.

MS. SHAPIRO: Would this revision of that sentence help? "These particular guidances address the need for subjects to inform their future contacts of their potential of their, i.e., of their, parenthesis, the contacts', "s" apostrophe, potential risks of infections from source animals? The need for subjects to indefinitely defer the donation of blood and other body parts and other issues," blah, blah, blah?

DR. VANDERPOOL: Thank you for clarifying. Eda.

DR. BLOOM: In fact, one of the FDA guidance documents, the one on donor deferral, does talk about deferral of the contacts, and intimate contacts.

DR. COOPER: So that is not confusing.

DR. BLOOM: Yeah, and you may recall that there are case-by-case decisions in that—and that case-by-case decisions are mentioned in that particular guidance document, for example, would be skin cell product that had ex vivo contact, our advisory committee didn't think it was necessary for close intimate contacts to be deferred from blood donation, if their contact received skin cells that had been grown on mouse feeder layers.

DR. VANDERPOOL: The next comment is at page 11, lines 28 through 31, at which point we say that "The first brief paragraph should specify the number of persons/participants to be enrolled in the potential subject's at the site, and the pertinent total number of persons." The question is, is it possible that "the total number enrolled" may give

some subjects a false sense of security? Will the information also include several survival rates of subjects who have undergone the same procedure, or rates of complication that have occurred is relevant because of the risks of these procedures inherent to xenotransplantation.

I think we say elsewhere that there needs to be a fully disclosed, however brief, history of how the procedures have—how effective or ineffective they've been in the past. So the question would be here, does giving a number give a false sense of security?

DR. MICHAELS: I concur. I think that the number has to be there, in that just as you mentioned, there should be disclosure of what has happened, and how patients have done. I mean I think that information needs to be included to help true informed consent.

DR. VANDERPOOL: I don't think would do layering, so the number is likely not to be high for a very long time, so I'm not sure a false sense of security would come from that.
Page 17, lines 13 through 14, "Is it legal to require an autopsy?" I thought it could be strongly recommended and requested, I am not sure it could be required.

MS. SHAPIRO: On Page 16, it is framed as an expectation, the autopsy.

DR. VANDERPOOL: Okay, page 21, lines 13 through 18, these are questions involving private physicians being at the forefront of—being at the head of the phalanx, so to speak, and therefore public health organizations need to stay in contact with them, and the comment here is the relevance of this is not clear. And in other examples of infection spreading to general populations from a xenotransplant recipient, no, they there are not such examples, but what about the first?

This comes up again later on, when, we are asked at the very bottom—toward the very bottom of the second page, item four, under "Recommendation: This is nebulous, which public health organization, "How should this communication be maintained between the public health organizations and physicians?" Comments about that from the committee or subcommittee?

MS. SHAPIRO: I think we meant "public health departments," actually, so that answers the first part.

DR. VANDERPOOL: So it should be after "organizations" the "Public Health Department"? Sharon.

DR. KIELY: I'm not sure specifically where you are when you're saying that? Are we referring to line—page 21?

DR. VANDERPOOL: Page 17, 13 through 14.

DR. KIELY: When you say "We were referring to public health departments," where is that specifically?

MS. SHAPIRO: Line 15, on page 21.

DR. KIELY: As opposed to "public health organizations"?

MS. SHAPIRO: Correct.

DR. VANDERPOOL: Further comments? Okay, line—

DR. MICHAELS: I have just a minor comment, one, I would change "private physicians" to "primary care physicians," minor, but I think that that might make more sense, and, two, I think that the sentence on—still on page 21, 16 through 18, the fact that, which is also one of the comments in here, particularly important in light of data suggesting public health organizations' ability to detect and monitor has declined due to false perceptions that such threats to health have decreased. I am not quite sure that still holds true. I think the public health perception of emerging infections has really been increased in the last couple years, with West Nile, SARS, what have you, so I think we could delete that.

MS. SHAPIRO: Yeah, this was written before that.

DR. VANDERPOOL: Thank you, Mary. I think that is a good suggestion. John.

DR. ALLAN: Getting back to the "private physician," the only thing I want to bring up there, the only thing in this whole document that really struck me is because the way health care has changed these days, what is a private physician or primary care physician? Many days now people just go in and see a doctor, and if you—if the person that received, like a xenogeneic cells or something, then it is going to be much less likely they would get caught through the system, rather than a whole organ, so I am worried that, you know, primary care physician is out the window in terms of many people now with the way their health care system is set up, they just go in and see a doctor, and they just go and they say, "I have a fever. I don't feel well and have diarrhea," and then the physician never gets the information that they had xenogeneic cells at some point in their lives, unless they are tattooed, or something, which I don't think is going to happen.

DR. VANDERPOOL: That is an excellent point. I think we should probably change the instance of "primary care" to "physicians in hospitals who first see patients," or use wording to suggest the variety and standards that you just recommended. Bill.

DR. SCHECKLER: Except that I spent my whole career training primary care physicians and populating the state of Wisconsin and elsewhere with them, and it's quite true that in different areas of the country there are different setups for processes of physicians, and you perhaps, since you don't want to delete the entire page 21, as I suggested, you want to fix it, I would say that the physicians and other health care professionals that take care of patients with xenotransplants is how you would put it, rather than use the word "private," but certainly physicians are going to be involved, and hopefully with electronic medical records and other kinds of processes that are both present and on the horizon, facts about the pre- or prior history of patients, that they might even carry a chip with them, will be more accessible than they are now, rather than less accessible, so I yet again disagree with you, John.

DR. KIELY: Can I just make one quick comment? I mean I agree with Mary, in that we don't have to say "private," and we can word it any way we want, "primary care providers," if we really want to recognize our nurse colleagues, and I think we should. But the point is that if you see a person who is sick, you should take a history. And the history includes these things, and if the patient is not going to be forthcoming about it, that is that. I don't think we can hedge our bets on everything that can possibly happen, but you would just hope that if a person presents with the symptoms that John has outlined, that they would take an appropriate history.

DR. VANDERPOOL: Good. We can work those comments into the report also. Yes.

DR. ST. MARTIN: I guess I'm still not sure if you are suggesting that public health departments provide some ongoing education to primary care providers, in terms of—What do you mean by "ongoing communication," or "lines of communication between public health departments and primary care providers"?

DR. VANDERPOOL: Definitely, we are recommending that.

DR. MICHAELS: Just to reiterate, that I think that we feel that as the public health providers have more information on what is going on in a national and international basis, that that information be disseminated to primary care, health care workers so that they are cognizant that patients that have received xenotransplants may be at risk for a new infection, and it has been recognized that they may not otherwise have the ability to know on their own.
So I think it is just a statement, really, that we continue to have ongoing education as we discover new information.

Can I make one other comment? I wanted to just comment about Bill's earlier statement about removing page 21 or page 20 and 21 together. While there are lots of infectious risks from different groups, I think that the fact that xenotransplantation is another route should not dissuade us from trying to minimize the risks of infection, so I would not want to remove this section.

DR. SCHECKLER: I'm just putting it in the science section, not in this section.

DR. VANDERPOOL: Moving along, we have a couple of points on page 21 with reference to a comprehensive survey is currently under way, and then a reference to the current federal regulations. I think the second one is done with federal—with the, very easily—Professor Shapiro, do you have a comment about the comprehensive review?

MS. SHAPIRO: Yeah. First of all, Bill, the Model Act that you talked about, that is just a Model Act, right? Bill Scheckler?

DR. SCHECKLER: Yes.

MS. SHAPIRO: So it hasn't been—it is not law. It has to be adopted by the states?

DR. SCHECKLER: It has to be adopted by the states. The states still have the public health statutes. There is never going to be a federal preemptive public health statute. But many of the states already have most of these things, like Wisconsin does, for example, incorporated, that they have incorporated in the last couple years, or in the last decade.

MS. SHAPIRO: And that is a good thing, no surprise that Wisconsin is out there in the forefront. But as an update on the second-to-last comment, one of my students did create a chart of all the current state public health laws that would be relevant to the issues that we are concerned about, and the next phase is to analyze that, in terms of applying it to where the gaps are, if any, in light of recent revisions, so it is ongoing.

DR. VANDERPOOL: Excellent. We are getting toward the end of—on the second page of comments, page 24, lines 14 through 13, we are talking about decision-making capacity, determination of decision-making capacity. This poses some concerns, these sentences, especially in light of restrictions. For example, intravenous drug addicts, or alcoholic is in full hepatic failure, and is homeless, do we suppose that person would be an ideal candidate for lifelong follow-up? My answer would be hardly. I would also think that during that clinical research phase, that we would propose that only those individuals who can give consent would be placed in a trial. The patient must give consent, not a physician, or relative, or family member. That is a more serious concern.

The Belmont report certainly assumes that. This is discussed at some length on pages 24 and 25. There is some federal guidelines regarding non-consenting patients. The Belmont report, which is the official ethical statement of the NIH and the OHRP, does say that non—that there can be proxy consent for research. But it outlines the conditions for that consent, which we state in our paper.

So the question here is should we say that there should be research only for those who can give consent, or should we leave it as we have it, where for the most part, patients should be able to consent, but if there is a possibility that they could come back to full comprehension, that we can conceive of certain protocols in which they might be enrolled. Comments?

DR. SCHECKLER: I should think the more prudent action would be to not include those that might become able to comprehend things in the future as recipients for—at this stage in the xenotransplantation experience. It would not be prudent. I can understand, just like, it is kind of like the issue with children and parents saying "You can't exclude my kids." You don't have that kind of barrier for this group, and I don't know, those of you that have actually thought about, or participated, the surgeons in the group and so forth, in terms of the kinds of patients, I wouldn't think you would want these kinds of patients in. On the other hand, maybe it is the desperately ill ones that can't consent that are most likely to be the subjects for the Phase 1 trials. It seems to me that it is really not a good idea to have these folks in, I don't know if you ought to leave that as an option or not.

DR. BLOOM: As Megan alluded to earlier, there have been patients with acute hepatic failure that have been treated with extracorporeal perfusion or a liver assist device, and those were comatose, and in some cases the literature reports that they had been bridged to transplant surgery. But they've had an assist type of device. A liver assist type device is intended for patients that may be in acute hepatic failure, and therefore may be comatose. I don't think that you can put a bar either way.

DR. SCHECKLER: Fair enough.

DR. VANDERPOOL: As the members of the committee and I assume, we spent quite a bit of time on this. The actual wording, Bill, we ended up using is on 26, lines 6 and 7. We recommended at this time enrollment for mentally impaired individuals should be limited to those in whom mental capacity is likely to be restored. We don't say "might be" restored. So I think we give—we try to give a verbal expression to say this should be a rigorous situation, you know, truly defendable.

The last thing to notice is page 26, the very last item, item five, of the recommendations, which reads "Legislature should evaluate the effectiveness of current public health laws to address situations which asymptomatic xenotransplantation recipients fail to comply, and symptomatic xenotransplantation recipient fails to comply with surveillance instructions, they should consider appropriate amendments to these laws." And this comment says, "Well, wouldn't this lead to inconsistency if the states do this? Why not recommend something from Congress?" Comments?

DR. SCHECKLER: Just—Again, I guess know more than I ever wanted to know about state public health laws. There are general statements, powers that are given to state health directors, state epidemiologists and governors that in most states in current law that would really be—that would subsume this kind of concern. And the structure, since it is state-based, the structure to go to a federal requirement like this would be really a different kind of precedent, as I understand public health statutes, and would have all kinds of barriers. So I don't think that is a viable suggestion. And I think, frankly, that you don't probably need number 5 here, in your recommendations, because I think that is already in the process being addressed, and it is addressed. You don't have to have a separate xeno clause in the public health statutes, because the public health statutes look at all possibilities for new and unrecognized diseases and entities that need to be followed that have a public health significance.

MS. SHAPIRO: It is state requested, I mean that would be my response to the general comment that that is the system we have, that it is state by state for the most part, that is how public health is regulated. And while I am hopeful, I am not quite as optimistic as you are, Bill, about the Model Act being the answer, in that there is no requirement for any state to adopt that. Another way that actually is something I've spoken with somebody about already to work toward a consistency state by state in laws like this. The thought, by the way, wasn't to have a specific xeno provision, but rather to have a paradigm that would fit xeno—but a uniform state law is also a way to go, and the Uniform State Commissioners on Uniform Laws is a body that has representatives from all states that are appointed by the governor of every state, and every now and then they will come up with a uniform law, such as the Uniform Determination of Death Act, which has been adopted by all states, and that, as opposed to a model state statute, is more likely to be adopted, and to provide consistency. And a commissioner, the head of the commission, is actually interested in a Uniform Act on this, at least, in terms of what he said to me, and public health wrinkles that have arisen, not on account of xeno, but everything else that we have been dealing with in society in the last year or two, so the answer, I think, is that we feel that it is still important to keep that in.

DR. VANDERPOOL: We have several categories in IRB deliberations, two of which are approved with conditions as stipulated, disapproved, and so on. I think we probably got a sense of a meeting on a couple of things, a sense of the committee on a couple of things. One is how many members of the committee are in favor of deleting pages 24 and 25? 22 and 23, is that it?

DR. GROESCH: Bill, was your suggestion, you made a very specific suggestion, beginning—

DR. SCHECKLER: I'm sorry, 20 through 23.

DR. VANDERPOOL: 20 through 23. How many on the committee are in favor of deleting those sections from the report?

DR. SCHECKLER: It is putting those issues in the science report, not this point.

DR. VANDERPOOL: Just for the sake of this report, deleting them, and we'll think about where they would go afterwards, but would be for deleting them from this report. All in favor, raise your hand (indicating). All opposed (indicating).

DR. VANDERPOOL: Okay. So I suppose we'll keep those pages here. Second would be how many of you are generally in favor of the—are in favor of this report with respect to its recommendations regarding research involving children? Let's see your hands if you favor what the report is saying on research involving children?

DR. MICHAELS: Could you just restate it?

DR. SYKES: Restate it.

DR. MICHAELS: Just please clarify what is the report specifically saying? It is not saying that you cannot perform xenotransplantation on children, it is saying that it should be done when the potential benefit is high, is that correct?

DR. SCHECKLER: It is recommendation number 10 on page 28.

DR. VANDERPOOL: "At this time, as a general matter, children should not participate in xenotransplantation protocols. There may be special circumstances in which the possibility of benefits to a procedure is high, given the available alternatives. Research institutions should consider these situations on a case-by-case basis, and should pursue further study of this issue." How many members are in favor of that recommendation and the discussion that preceded it?
Raise your hand (indicating). How many opposed (indicating)?

Okay, so I think we are ready for a motion, and if there is such from a committee member, that this report would be approved with the conditions that we've specified, and clearly written down, many of which are—all of which—well, many of which were accepted as editorial comments. But, is there a motion that the committee would approve this document as amended, so that it would be ready for publication and public comments? This motion would include our doing the final workup on the report, and getting it back to you, but unless getting it back you to, but if you had particular objections or something, I suppose we'd have to come back. But let's make—Would anyone like to make a motion for approval of this report?

DR. MICHAELS: Can I actually clarify one other section before we have the motion? I know that we did discuss the issue regarding the inability for an incapacitated adult or child to give their own consent, but the one part that I don't think we distinguished was the part of having a proxy—having a family member, or make that consent versus on page—the end of 24 and page 25, the section where the researchers could make the decision, and I just wondered if there was any further discussion on that? I was a little uncomfortable with not being able to have any kind of consent given outside of the researcher's advice, if I understand this correctly, particularly in lieu of the fact that once the xenotransplant has been performed, you can't withdraw, in terms of removing the xenotransplant—

MS. SHAPIRO: The exception for getting consent from a legally authorized representative applies only in an emergency situation, and is general federal regulation. So is your question should we advise that that opportunity should not apply for xeno?

DR. MICHAELS: That is what I would recommend at this point.

DR. KIELY: Robyn, would you reiterate that?

MS. SHAPIRO: Yeah, what is on the bottom of page 24 and the top of page 25 talks about when you do not have to get permission from a legally authorized representative to enroll an incapacitated individual in research, and that applies only in emergency situations. So the suggestion is that we not allow for that to happen, although it does in federal regulations, generally, we not allow for that to happen if the research is the xeno.

DR. SALOMON: I know there has been testimony from our heart colleagues that such a stipulation would be a problem, in that one could conceive of a situation in which there really was, and I'm not saying it exists this minute, but that there really was a successful intervention, let's say for a child with heart failure, and it would be wrong, I think, to officially prevent such a thing from happening. It would also run—I think you already pointed out against the typical flow of that through many other federal regulatory committees and advice.

DR. ALLAN: What about if you say "at this juncture," or "in the early stages," because we can always re-visit that, if there are—if they get ready to do heart transplants that are, you know, that may involve these kinds of situations, then we can re-visit it.

DR. SALOMON: You know, at a certain point, you got to stop here and say, you know, these are doctors. They are ethical. They care about their patients, they've got institutional review boards, and everybody looking down their throat. We don't have to do everything.

DR. KIELY: In my view, it's taken us this long to get this far, John, and to make additions and public comment, I mean this is law, and I don't think we should strip physicians, and many of whom are researchers, of what is considered to be the right of the patient, as well as the institution to do what is in the benefit of the individual patient.

DR. BLOOM: If there is any additional concern about that type of situation, please recall that Epicel, which I keep referring back to, is the skin cell product in which human cells can co-culture with mouse cells, it's a xenotransplantation product. That product is intended for use in patients with burns, I think it's 30 percent of their body, third-degree burns, and that may be a decision that is made very rapidly. You may not have the ability to get consent from a patient, at least before you get the biopsy.

DR. VANDERPOOL: Ellen.

DR. GADBOIS: Thanks, I'm sorry. I just wanted to point out that I'm not sure if it's meant to be this way or not, but it sounds like there is some confusion of research and treatment terms. This is a provision that applies to research, and federal regulation, not treatment.

MS. SHAPIRO: While you were out of the room, we talked about whether or not we wanted to change the bottom of page 24, top of 25 by saying that you could not apply the research emergency provisions and federal law to xeno, so maybe we should get a sense of the committee about whether we should change what is currently written, which buys into it, or not.

DR. VANDERPOOL: Is that okay? That is a big issue. I'm not sure we need to deal with that in the informed consent document. it seems to me that issue of—we've outlined pretty clearly where consent is to be procured, and in what conditions. It seems to me that to endorse or prohibit xeno in the light of these ER regs raises a set of issues that would leave our subcommittee to go back to and hash out over time, but maybe I'm wrong about that. Further comments of the group?

DR. ALLAN: I would just say I would like us to vote on that one particular issue, that way at least we have a minority opinion versus a majority, depending on how the vote comes out, and that way we can move on. Because at some point you are going to want to take a vote on the whole document. And if there is certain things that are outstanding that are somewhat contentious, if we vote—if we could vote on the individual ones, then that is already documented, and then we could go to the next one.

MS. SHAPIRO: All right, anybody—We will just vote on whether or not we should change what is currently written on the bottom of page 24 and top of page 25 in a way that would recommend that the emergency research provisions and current federal law not be applicable to xeno recipients and protocols. So all in favor of changing what we have now? (Indicating.) One, two—And you would do what I said, you would say that even under the emergency circumstances, that the provisions that would allow participation for other kind of research could not allow participation if it involves xeno. Okay. All who vote the other way? (Indicating.)

MS. SHAPIRO: Okay.

DR. VANDERPOOL: So are we ready for a motion, approval with the stipulations as we've outlined them over the last hour and a half?

DR. COLLINS: So moved.

DR. VANDERPOOL: Dr. Collins, so moved.

DR. MICHAELS: Second.

DR. VANDERPOOL: Is there a second to that?

DR. MICHAELS: Second.

DR. VANDERPOOL: Is there a discussion?

DR. KASLOW: Yeah. I would just like to see if you sort of have some ideas about what you are willing to consider further in the way of changes in the document before we call it final, and it's posted as final.

DR. VANDERPOOL: Thank you, Dick. Robyn has been taking notes. You will be passing in, if you have editorial changes, what I am assuming at this point is that Robyn, myself, and Mary will go through these very carefully in the spirit of the transcript of this meeting, make sure that our changes accord with the suggestions that seem to have group approval, and that we make all of those changes, and that at that point we would send this document out to the entire committee, and—as a finalized document. If someone says, you know, you should use a semicolon here instead of a comma, or a different word, such as we have been—has been suggested, then we certainly would do that. But what we are wanting, what we are striving for, Dick, is an actual action on behalf of the committee, namely, that we have tentative approval of this document under the conditions I just specified. Does that clarify things?

DR. GROESCH: And just to clarify a bit further, that we are talking about getting it to a point where it could be posted for public comment. It is not to be the final document that would be submitted to the secretary.

DR. KASLOW: I see, okay.

DR. GROESCH: Then we would come back and there would be more discussion, in light of public comments received about what final changes.

DR. KASLOW: Okay, I guess, as an intermediate step, or an alternative step, and I'm not necessarily saying I favor it, but let's just discuss it for a moment, would be for you to make all of the changes, and highlight them in the document for us, so that we can see those particular changes, and how you've worded them. Let us take a look at that, and then make a final approval decision based on that form of the document, rather than trying to do it today, without our really understanding what the wording would be.

DR. GROESCH: Yes.

DR. VANDERPOOL: That is certainly acceptable.

DR. KASLOW: I don't know if that is an amendment, or a suggestion that we disapprove your motion and make another motion, but whatever it is, if we could get that kind of modification in, I think it would be helpful.

DR. VANDERPOOL: Okay, we'll ask Dr. Collins does he wish to withdraw his motion at this point to see how this second motion flies, or not?

DR. COLLINS: I am willing to withdraw.

DR. VANDERPOOL: Okay. Dr. Kaslow, do you have your own motion for the floor?

DR. KASLOW: I'm not sure I have it formulated explicitly, but let's just say that we would—I would move that we review a document that contains all of the suggested changes, whether they were going to be handed to you after the fact, or have been discussed publicly now, that you incorporate those into the document that you will then circulate to us with the specific highlighting of those changes suggested, and that we can then vote on those changes,

or discuss those changes, but vote on the entire document, if necessary, as well. I know that is a complicated motion.

DR. VANDERPOOL: Said like a senator. Is there a second to that motion?

DR. SALOMON: Can I do that? You told me I could vote.

MS. SHAPIRO: Sure, I'll second that.

DR. VANDERPOOL: Second. All in favor, let it be known by raising your hand (Indicating).
All opposed? (Indicating.)

DR. VANDERPOOL: It has been passed, and we shall take a break.

DR. SCHECKLER: I would like to ask the indulgence to get the voting members, as well as the liaison members, just stay where you are—the voting members in the front, stay where you are so I can get a couple of pictures, if anybody is willing to have their picture taken after this morning. But I'd like two pictures, one of the voting members, and just one behind this group, and then with everybody right now.

<BREAK>

DR. VANDERPOOL: Let's take our seats and move to the rest of the program. Thank you for coming.

DR. GROESCH: Okay. We have a series of scientific presentations, and our first speaker is Dr. David Cooper of Harvard Medical School, and he is going to be talking to us about recent progress in pig-to-baboon heart transplantation using hDAF, and alpha-13 galactosyl transferase gene knockout pigs.

Agenda Item: Recent Progress in Pig-to-Baboon Heart Transplantation

DR. COOPER: I'm going to just give you some information about what we have been doing at Massachusetts General Hospital for the last two or three years in the field of heart transplantation. You'll remember that the pigs that have the expression of hDAF, the human decay accelerating factor, have some protection against human or baboon complement activation. I'll come back to that in a minute, and pigs that have the alpha-galactosyl transferase gene knockout, they do not express this Gal epitope, which is the major target for anti-pig antibodies. So they are protected in a different way from this hyperacute rejection process. Now this is clearly a work of a large group of people, including many fellows and technicians at the Transplantation Biology Research Center, which is directed by David Sachs. But also a large number from Immerge Biotherapeutics, which is, as you know, a biotech company with which we have been working very closely, and I'll perhaps mention some of their contributions as we go through.

Now just to put you in the picture again, a major barrier we believe for the last 10 years or so to the successful transplantation of pig organs into non-human primates or into humans, has been the presence of natural antibodies, which we develop a few weeks after we are born in primates and humans directed against this Gal sugar epitope, which is present on pig vascular endothelium. And in the first study that I'll briefly review, these hearts were taken from pigs that had this transgene, a gene inserted for human decay accelerated factor. Pigs have some protection against their own complement, but it provides very poor protection against complement of a different species, including the baboon. So these were transgenically manipulated pigs that expressed, a complement regulating protein that protected them from immediate complement activation.

These pigs were developed by David White, who is going to be speaking to us a this afternoon in Cambridge in Britain, and were the first major contribution and development to the field in the respect of manipulating the pig, rather than trying to immunosuppress, or manipulate the baboon. So they were a major step forward, and they took us rather further, I think, than we thought we would get at that time, certainly a first leap in xenotransplantation. But we also, because these pigs did express this Gal sugar, we also gave them part of their immunosuppression regimen, we included the continuous infusion of a Gal conjugate, that is a synthetic sugar, sugars that mimic the Gal epitope to absorb anti-Gal antibodies, and I'll come back to this in a moment.

In the second study I'll briefly present, hearts produced by Immerge Biotherapeutics, in which the gene for the enzyme that makes this galacto sugar, which is deposited over the vascular endothelium, the gene was knocked out by a nuclear transfer technology, so that these pigs do not express this major target for anti-pig antibodies.

And we used these organs, transplanted into baboons using the same immunosuppression regimen as in study one with one or two changes. For example, we did not need to infuse these Gal conjugates, because if there is no target for the anti-Gal antibodies from the baboon, on the pig, then obviously we don't need to block them. They are going to be innocuous. So there were some changes to the regimen, but basically the regimens were the same.

And just to go over this again, this is the lining of the pig blood vessel, the pig vascular endothelium. These are the Gal sugars that stick out from the lining, and you could see the human or baboon antibodies bind to these, and then they activate complement, and the complement punches a hole in the cell to destroy the cell. And we'll see in a minute how those have been modified. The technique we used was to put the pig heart into the abdomen of the baboon so that the basic advantage of this is that when the heart beats, if it is rejected, you can obviously tell it is rejected, because it stops beating. But afterwards, you can take this heart out, and the baboon will stay alive. It is obviously technically much easier than putting the heart into the normal position and taking the baboon's own heart out. The advantage is you can follow the baboon after you remove the heart to see what happens to, for example, antibody levels, and so on. So it is a fairly simple technique that we carried out in both of these studies.

The first study is this one where we used pigs transgenic for this complement regulating protein, and we infused the sugars to bind the anti-Gal antibodies. Here is an example of here you see the Gal sugar, just as before, but the synthetic sugars here are being infused continuously into the blood, so that the antibody largely binds to the synthetic sugar, and therefore cannot bind to the sugar on the pig organ, and therefore this protects the pig organ from the effects of antibody binding and subsequent complement activation.

And you could see how effective these sugars are. These sugars were made by Novartis, the pharmaceutical company. And others have tested these as well, but you can see that if you measure a subgroup of this anti-Gal sugar, the anti-Gal antibody, IgM, and another subgroup here, and then IgG down here, you can see as soon as you infuse the sugar, you get virtually no antibody measurable in this subgroup, and very low levels here in this subgroup. And we think these low levels that still persisted here were very—antibodies with very low affinity so that they didn't bind very well to the pig organ for one reason or another, because even if we increased the amount of sugar being infused here, we still have this level here. So they were not binding to the sugar very well, but they probably weren't binding to the organ either. So largely we effectively removed the effective antibody, and we also protected from complement activation, in case some antibody did bind.

Now this is the regimen we used. It looks rather complicated, in fact, it is rather simple. We treated the patients—the baboons with anti-thymocyte globulin, which knocks out your T-cells, which are the things we tend to knock out in humans, because they are the most effective anti—the most effective rejection cells in humans. And we also used an anti-CD2 monoclonal antibody, which Immerge provided for us in case the ATG didn't do its effect well, you still have a number of T-cells after the transplant, which from day zero, we gave a little bit more, so we knocked out the T-cells, which is standard treatment in many standard regimens using humans. We also gave some thymic irradiation, which knocks out T-cells. This we believe is no longer necessarily, but was a hang over from our bone marrow transplants previously, but in one experiment, we did not use this, and it did not seem to have much major effect.

We also used the immunosuppressive drugs mycophenolate mofetil, which is used in humans, but we used it in a very low dose, very low dose, and we used steroids we thought for this low, a tapering dose, and by the time we got up to about four weeks, you were on the sort of dose you might give to a patient, a human patient. One of my colleagues has tested without this in the regimen, and this doesn't seem to be necessary either, se we are down to a pretty low level of immunosuppression. But we did use an anti-CD154 monoclone antibody, which blocks the effect of these remaining T-cells. And this is a drug also made by Novartis, which we believe is very effective in blocking this T-cell response, so most of this immunosuppression was geared towards blocking T-cell activation, which is the major cause of rejection in human—to human transplants.

We also gave heparin to anti-coagulate the blood for various reasons. We thought this was important. Prostcycline has some effect on the endothelium, it stops it being activated, and so on, and we—in addition to having the human complement regulating protein on the pig organ, we also gave a complement inhibitor, or a complement depleter such as cobra venom factor, just to be absolutely sure that the complement was not going to play a role. So we covered pretty well all our bases here in this regimen. And just to draw your attention, here is the complement regulatory—the complement inhibitor, and here is the sugar being infused. We used two different sorts, but this is not very important, and you can see in this pig, in this baboon, we did not give any heparin. And in these, we gave a lower dose of heparin starting on day two, and here we gave a higher dose of heparin starting on day zero, immediately after the surgery. So these were the major differences between these sort of subgroups, that we heparanized better. And here you can see the partial thromboplastin. I am showing that that second group, subgroup, brought a higher level of anti-coagulation than the first group, which may be important.

Now if you look at the T-cell responses is a mixed leukocyte reaction. And you can see that before the transplant, before any immunosuppression was given, the blue bars show that this baboon, this is a representative one, this baboon had a high response against pigs of various types, including the hDAF pig, and a reasonable response against another baboon, but no response, of course, to itself. But once we gave the immunosuppression here on day 19, and here on day 38, you can see the response to all the pigs and to the baboon is very, very low, almost the same as self.

So this immunosuppressive regimen clearly suppresses that T-cell response very, very effectively, which is what we planned.

Now how did these experiments do? Well, if we exclude this one, which was the one that we didn't give any heparin to, and this one plotted the entire graft on day three, and we had to take it out, and that is what really made us think this heparin is important, that is why subsequently we gave more heparin. If you exclude this one that we believe had an infection before the transplant, and died of a pneumonia on day seven, and we have pretty good evidence that this baboon was sick beforehand, but unfortunately we went ahead with it, but if we exclude those two, you can see these first four survived a median of 22 days. The median is considered a better indicator than the mean.

And these four that only had extra heparin survived twice as long, to 54 days. Now, there is always a learning curve with these, so it may be the first four we didn't manage quite as well as the second four. You always have to bear that in mind, but the most important factor was the extra heparin. And we got a significantly longer survival out of these second four. So this was pretty good. Fifty-four days as a median is fairly good in this model.

And here you can see that these two that we excluded, at four and seven days, and then the earlier ones that got primarily the typical acute human xenograft rejection, they got some hemorrhage and interstitial edema, which was clearly the result of antibody. They also had a thrombotic microangiopathy, I'll show you in a minute, where you get little thrombi in the small blood vessels in the heart; whereas, the longer surviving ones had the reverse. They had predominantly the thrombotic microangiopathy, and minor effects of the acute human xenograft rejection, so we seem to change the pattern here from the typical rejection to a very atypical rejection, primarily a thrombosis going on in these small vessels, which we are not sure of the exact cause of that as yet.

And here is an example, this shows some hemorrhage and edema with thrombi here. Here is a fairly big one, whereas in the later ones, there was very little hemorrhage of thrombi—hemorrhage and edema, but a lot of thrombi. So it seems that you clot up these little vessels, probably something to do with endothelium activation, which causes a procoagulant effect here, and then you get ischemic injury around these vessels, and eventually the heart ceases functioning because so much muscle has been damaged because it doesn't have a blood supply. But a different picture from before.

Now finally in the one that went 139 days, we saw typical features of chronic rejection, which occurs commonly in patients, usually after a few years, with a human heart, but here we are seeing it after a few months with a pig heart. But I would remind you that in the early days of allotransplantation, this picture was seen within a month in many of the early patients, so it is not surprising to see it at this stage in the pig. And it doesn't mean to say that we can't eventually prolong this significantly.

Now what was important here, people have said, "Well, you are overimmunosuppressing these baboons. This is why you are managing to keep them going. They are getting a lot of immunosuppression, but I put forward to you that they are not getting a lot of immunosuppression, because the one feature of overimmunosuppression is you get a lot of infection. And here we see one baboon I mentioned died of pneumonia, which we think was present before the transplant. We drew blood cultures every two—twice a week in all the others, and you could see survival was from several days to 139. We drew more than 120 blood cultures, and only two blood cultures were positive. Both baboons had normal white counts at the time, suggesting they didn't have clinically infection, and the blood cultures became negative after we changed antibiotic therapy. No this is a very low incidence of line contamination, I wouldn't even call it infection; and therefore we can't possibly be overimmunosuppressing these baboons if this is the only so-called infectious complication we had in these 10 animals. So I think what we are using here in immunosuppression is the sort of immunosuppression you could easily use in a patient with good results.

So in conclusion, in this first trial, is that the Gal conjugate maintained low levels of antibody, the anti-CD154 monoclonal antibody based therapy was associated with inhibition of elicited antibody. We didn't see any new antibodies coming out against non-Gal antigens, for example. We know that if you put a pig organ in, you develop lots of new antibodies that weren't there before, because the baboon sees these new targets on the pig organ, and makes lots of new antibody, but we didn't have any evidence for any of those.

We clearly suppressed the cellular response, which is the cellular response you see in a human to human transplant, because the MLR was flat all the time. And we didn't see any cellular infiltrate of the graph, suggesting that the type of rejection we see in a human organ transplant was completely blocked by this regimen. So graft failure was really associated with these fibrin/platelet thrombi that occurred, which could be due to low level of antibody attaching to the graft that we couldn't detect, activating the endothelium, and causing a procoagulant status, instead of an anticoagulant state. Clearly all of our blood vessels and all of the pig's blood vessels normally have an anticoagulant state locally, otherwise the blood would clot on them. But this probably was changed in these situations, and fibrin was deposited, and platelets got stuck in them, and got fibrin/platelet thrombi, causing these obstructions. The higher dose of heparin appeared to prolong graft survival, presumably by slowing the effect of this fibrin/platelet thrombi, and when we got out as far as 139 days, we can associate this also with a low rate of infectious complication, suggesting that this immunosuppressive regimen is perfectly—would be perfectly acceptable to a human patient undergoing this procedure. So now let's go on to the second brief study where we use these hearts that were from pigs in which this galactose was not present anymore, genetically modified pigs, which do not express this any more, so there is no target now for this key antibody, anti-Gal antibody. Now the regimen was very similar. The only significant change is we didn't need to infuse the sugar all the time, because we didn't need to block those antibodies. Some of the experiments did not give cobra venom factor, because we thought, well, if there is no target for antibody, we are not going to activate complement.

Otherwise, the regimen was almost the same, if not identical, to the previous one. So it is a good comparison between the two. And we did eight transplants in this group so far, and we use also as controls here two pigs that, although not wild type, did still express some Gal on the myocardium. And right up front, I'll say now that those both hyperacutely rejected, having the same regimen as the others. They hyperacutely rejected within minutes, within 20 minutes in both cases. So clearly, if you don't—if you still express some Gal, this regimen is not going to be very effective, and you lose all your grafts within minutes, so that is a good comparison with these eight that had Gal knockout hearts. And you can see in some we didn't give cobra venom factor. In some we tried an antithrombin, which is a type of anticoagulant which we thought might be valuable, and in three of them we also gave aspirin orally, because aspirin prevents platelet activation and platelet adherence. We thought this might help in preventing this fibrin thrombi that we see. So there were some minor, minor variations. Again, you can see this is the response to the pig cells before the transplant, the T-cell response in blue, very high. Once we give the immunosuppression, it is knocked out completely, and the dark red bars here are about 43 days after we stopped all the immunosuppression. This graft survived 110 days, and we stopped all the immunosuppression there, and you can see 43 days later there is some beginning return of anti T-cell activity. As before, very good suppression and good T-cell response. And here is the survival. You can see that two we had to euthanize 225, 226, one for anemia, we didn't have any blood, unfortunately, to give it at the time, and the vets felt we should euthanize it. And one because it got a thrombus in its leg associated with a catheter, we think, that was in its leg. So these two were euthanized for unrelated reasons. Their hearts were still functioning well.

And this one died because perhaps the heparin was a little bit too high. And it died of a hemorrhage in its abdomen, which we really couldn't account for, but it was doing well. The heart was doing well at the time.

Of the remaining five, you can see that two have got more than 100 days, and the others all got to more than 50 days. And this one is still ongoing today, is 110 days, so we've got to—he will be the longest survivor in this group. So we are doing significantly better, I think. And again, in some of those that survived about 50 days, we did see features of acute humoral xenograft rejection, but predominantly again the major feature in all of these hearts is this thrombosis occurring in these small blood vessels.

You could see all these small blood vessels with a thrombus in, so that the myocardium will eventually becomes ischemic. This started fairly early in most of them. By day 16 we saw it. And in the one that we already euthanized, or whose graft we took out at day 110, had the same feature as this chronic rejection as we saw previously.

And we believe that anticoagulation at this stage is a major important factor in the treatment of these animals, because to try to overcome this thrombotic microangiopathy, and you can see they all had high dose heparin, as the previous group had, but this is clearly not enough to stop the fibrin thrombi occurring, because they all got the thrombin. The antithrombin, which we only had available for a short course, so we didn't give it a really good trial, didn't seem to affect the incidence of the fibrin thrombi. But I'm glad to say that just adding a baby aspirin every other day made a significant difference. This is baboon 228, who is now day 110, and this is a biopsy on day 95, and it is absolutely normal. There are no effects of thrombotic microangiopathy, and we have two previous biopsies also, which were normal. So here we have a baboon out to nearly four months with a normal myocardium, and the only significant difference we've added to this regimen is that we've added aspirin to try to prevent these plated thrombi, so I think we're getting to the point where we got at least even just one animal with a pig heart functioning well nearly four months afterwards with completely normal myocardium, and no signs of rejection, with a regimen that is very easily tolerated by these animals, I think we are making some significant progress.

And to reiterate on how well tolerated it is, in this group of eight animals, no baboon suffered any morbidity from infectious complication. Blood cultures were again drawn at least once or twice a week. More than 100 blood cultures were taken in these eight baboons, and they were all negative, except for three in two baboons, and they showed these organisms. Both became negative after antibiotic therapy. These were just routine draws from the lines. These baboons have intravascular lines in continuously throughout this whole period of time, this four months, whatever it is, it is not surprising that occasionally the line will get some contamination. But none of them were sick with this, and the blood culture became negative when we were changed that antibiotic therapy. So again, no signs that we are overimmunosuppressing these animals, because they are not getting any infections.

So how can we compare these? Well, the Gal—these five that we can assess carefully, the median now is 78 days, and you'll remember in the best of four of the subgroup of the hDAF, the medium was 54 there, so we've made another jump here to 78 days. And 78 days is not too far from three months, a consistent three months. So we are getting to a state where we think that with a few more of these, we might be able to do some orthotopic transplants, and if they went for three months, we think we are making some significant progress.

So in conclusion, in that group, no hyperacute rejection was observed, even without cobra venom factor. The Gal knockout heart grafts functioned longer than heart grafts from the hDAF pigs in the absence of therapy aimed at reducing natural antibody or complement levels. Fibrin platelet vascular thrombi were the major pathologic features in these heart grafts, in all the groups, really, and we're not sure if this is an immunological process or is it just due to molecular incompatibility between the coagulation factors of the pig and baboon. This may be important. But importantly, the antiplatelet therapy with a simple aspirin certainly delays onset, I wouldn't say yet it prevents it happening, because we haven't followed it long enough, but it certainly delays it very significantly.

So I leave you with this thought by a former chairman of the Rolls Royce Company, there are many ways of losing money, women are the most fun, gambling is the fastest, and research is the most certain, and this I put up just to show you that unfortunately we still need more research. But I think that we are making very significant progress in this field at last after quite a long period of time.

Thank you very much.

DR. GROESCH: We have another speaker, and then a brief discussion period, but if there is some specific questions that you just wanted some clarification on right now, you can go ahead.

DR. SWINDLE: Yeah, obviously you have chronic catheters in these animals with that kind of therapy.

DR. COOPER: Yes.

DR. SWINDLE: Are they externalized, or are they subcutaneously or implantable?

DR. COOPER: No, they are externalized. They come through an tether system to the top of the cage, and so that every time when you draw blood or give a drug, they are open to risk of potential infection.

DR. SWINDLE: And towards the end you slightly alluded to something that occurred to me while I was looking at these, and one is just the problem of the complications and chronic catheterization. For instance, were these thrombi also found, say, in the liver, spleen, kidneys?

DR. COOPER: No.

DR. SWINDLE: So it was strictly limited—

DR. COOPER: Yes.

DR. SWINDLE: —to the host organs?

DR. COOPER: —to the transplanted organ, yes.

DR. SWINDLE: You are positive cultures were taken out of the external lines?

DR. COOPER: They were taken out of the lines.

DR. SWINDLE: So it could have been biofilmed.

DR. COOPER: We sometimes try to confirm we are taking blood direct from a vein, but these are from the lines, and my feeling is that in the absence of any clinical features, or any rise in the white count, these were contaminants of the lines, maybe even just the technique of the person that drew it on that day and I don't believe these were—

DR. SWINDLE: Yeah, so these were external tethers going to the top of the cage?

DR. COOPER: Right.

DR. SWINDLE: It is quite likely you do want a biofilm.

DR. COOPER: It is actually a credit to the fellows looking after these that they had so few line contaminants, I think, because every day they are drawing blood or giving drugs, connecting up an infusion to these lines several times a day, so I think they looked after them remarkably well, but it emphasizes the point that these animals cannot be overimmunosuppressed, otherwise they would be having a much higher incidence of infection.

DR. SWINDLE: Okay, thanks.

DR. VANDERPOOL: We have several other questions, Dr. Scheckler, Dr. Michaels, and then I have a question.

DR. SCHECKLER: Did you study the pig's porcine endogenous retroviruses at all?

DR. COOPER: I'm sure Clive will speak to that this afternoon. We know they all have endogenous retroviruses, so I'll leave that to him. We certainly, in these two experiments, we made sure the pigs did not have

cytomegalovirus. Previously, though, we had no sign that cytomegalovirus was transferred to the baboon. And also we know all these baboons have baboon cytomegalovirus, but that the level of it was not up-regulated. Now in her over immunosuppressed, or heavily immunosuppressed individual, you up-regulated, so again, another feature indicating that we are not immunosuppressing these animals very seriously.

DR. PATIENCE: Just to confirm, David, this afternoon I will be presenting the PERV analysis on both the donor animals and the recipients.

DR. MICHAELS: I was actually going to ask about the viral infections, but I'll wait till Clive presents this afternoon, just because it is T-cell immunosuppression.

DR. VANDERPOOL: Dr. Cooper, my question, the first one, is overly naive, the next one hopefully is reasonably intelligent. First, now, did you keep the hearts of the—of the monkeys, and just have this implanted heart as an extra heart in the abdomen?

DR. COOPER: Correct.

DR. VANDERPOOL: Okay. And so my next question would be one of the concerns in our state of the science document is the degree to which there are cellular and—there are various physiologic incompatibilities. So with the functioning, heart still intact, you probably wouldn't see the extent of these incompatibilities, blood clotting or bleeding disorders, or whatever.

DR. COOPER: No, I think you would do, because in previous experiments, which I haven't presented here, we have seen all these factors. It is related locally to the organ, the transplant. For example, if you have severe blood clotting in the organ, you may lose your clotting factors in the blood generally. But if you take that organ out, you rapidly regain your clotting factors, so it is all related to the presence of the organ, and we did not see those type of complications here, with the exception of this thrombotic microangiopathy. It did not have a systemic effect.

DR. VANDERPOOL: Could you comment somewhat generally on the degree to which our lack of understanding of the physiology, even at the cellular level, is—represents a very significant barrier, or not so significant barrier for xenotransplantation at the present time?

DR. COOPER: Well, my—David White's group did some work on this, and it is very difficult to decide is the physiological nonfunctional, poor function, related to injury from rejection, or is it related to an incompatibility? With something as simple as a heart, I think if you—the evidence suggests that if you can prevent the rejection, the heart functions very well. Now, we would have to confirm that by putting the heart in the orthotopic position, and making sure it supported the life of the baboon.

But with kidneys, kidneys will, obviously, are life-supporting, and we think that the changes that are occurring are mainly related to the—a remnant of rejection, or something like that, not a strict incompatibility that would prevent functioning, but that obviously hasn't been absolutely confirmed yet, because we haven't had baboons living quite long enough with kidneys to be sure that there is no other physiological problems.

DR. VANDERPOOL: What about anemia, did this appear at all?

DR. COOPER: Anemia?

DR. VANDERPOOL: Uh-huh.

DR. COOPER: These all get a little bit anemic, because we are drawing blood every day, and so on, so they run at a little bit lower level than usually, but for example, the one doing that is doing well at 110 days now, and did not get thymus irradiation, this one has been not anemic at all, and hasn't required any blood transfusion, despite the fact we draw blood every day, so this immunosuppressive regimen is not suppressing the bone marrow to the point where they are getting anemic.

DR. SWINDLE: Are you monitoring physiologic function on this heart using echo? Is that—

DR. COOPER: No. We palpate it every few days. We also have a blood pressure monitor in it so we can see the pulse it is putting out. It is pumping out a pressure significantly above the diastolic pressure of the baboon. And we take biopsies at monthly intervals. So we are not really doing physiological monitoring, as you suggest, but we are keeping a pretty close eye on it, and we've seen no deterioration in function.

DR. SWINDLE: You don't see a gradual stepdown over this two or three months.

DR. COOPER: In this one, we haven't at all. But in some of the others, we did see a gradual stepdown before they rejected. But in the absence of any sign of rejection on biopsy, we haven't seen any change in function.

DR. KASLOW: What is the anatomy of the connections of that the second heart has to the rest of the animal?

DR. COOPER: The heart is—the donor heart, aorta is anastamosed to the aorta of the baboon, so blood from the baboon goes down the pig aorta into the coronaries, circulates through the coronaries, back into the right atrium. There is also a connection, we make an atrial septal defect, so blood from the left ventricle atrium also drains across, and then from the right side of the heart, it drains through the pulmonary artery into the venous system of the baboon, so it is not a heart that is actually carrying much of a workload, but in allografting, it has been a very good model for demonstrating whether or not the immunosuppression you are giving is going to be adequate to maintain good function, so allograft is a well-tried system and allografting has been shown to correlate very well with long-term survival, even after orthotopic transplantation.

DR. GROESCH: Thank you very much, Dr. Cooper. Our next speaker is Dr. David Sachs from the Transplantation Biology Research Center at Massachusetts General Hospital, and he is going to be talking to us today about an approach to xenograft tolerance using gal-T knockout pigs. Thank you, Dr. Sachs.

Agenda Item: An Approach to Xenograft Tolerance Using Gal Knockout Pigs

DR. SACHS: Thank you. I see that I have minus five minutes for my talk. I trust I'll be able to go ahead.

DR. GROESCH: Yes, please do.

DR. SACHS: Well, there are two actual approaches that we have been taking to xenotransplantation in pig-to-baboon model at Mass. General Hospital, and Dr. Cooper has just told you about our first one, which uses standard immunosuppression, and the progress that has been made, and the other major approach has been the attempt to induce tolerance to the transplant.

And the person who has been predominantly responsible for these studies, this has been in the kidney model, as opposed to the heart, has been Dr. Kazuhiko Yamada, an outstanding surgeon who has made a lot of technical innovations, which I'll discuss as well during this presentation.

As for Dr. Cooper's presentation, you see that this is a very large joint effort between many—I've mentioned here three of the major contributors from the Transplantation Biology Research Center at Mass General, but the "et al." applies. A large number of others who have contributed. The same is true from our major collaborator, Immerge Biotherapeutics, who again have an enormous input in these studies.

"Tolerance" is, by definition is the specific absence of an immune response to an antigen, this begs the question of how you arrive at that specific absence of immune response, but the method that we've utilized predominantly in our laboratory over the past quite a few years has been one called mixed chimerism, which involves a mixture of bone marrow elements, which leads not only to a chimerism of the bone marrow, but also to tolerance to any other organ from the same donor. That was first demonstrated in mice, mixed chimerism, by reconstituting a lethal irradiated mouse with a mixture of bone marrow from the host and donor type, and we found that you could get long-term tolerance, you could get long-term mixed chimerism, and long-term tolerance in any tissue, even skin from the donor strain of mouse. That has subsequently been taken to large animals, to pig-to-pig, and monkey-to-monkey and even now human-to-human, and I think it is important to point out that tolerance is now no longer just a laboratory model, but actually has now reached the clinic, and in fact I'd like to just show you a couple of slides

from the study also going on at Mass. General Hospital, and under the sponsorship of the Immune Tolerance Network, the ITN from NIH. And I'll just show you one patient, the first patient treated in that protocol who a renal allograft across a full hepotype HLA mismatch using mixed chimerism as the means of inducing this kind of tolerance. Now the actual protocol similar to most of the protocols that we'll be talking about today, and indeed even the one that Dr. Cooper initiated, is the standard immunosuppression with, involves T-cell depletion at the outset, to get rid of the mature T-cells in order to allow the chimerism to occur, and for tolerance to be induced. And then in this case, cyclophosphamide, a thymic irradiation, and a bone marrow transplant at the same time as the kidney transplant.

Now this particular patient wanted the protocol, because she had already failed the transplant from her mother previously, and that transplant had rejected on standard immunosuppression. But during that period that she was on the immunosuppression, she had had a terrible problem with warts as a complication of the immunosuppression. So she didn't want to have standard immunosuppression again. She really asked if she could be the first patient for this tolerance protocol. So by the time she rejected her second kidney, and had a creatinine of six, she came in for another transplant, this time from her father, and here is her course. Here is her cyclosporine, which is tapered. I don't know if I showed that in the first slide, but there was cyclosporine, which was to be done at the time of the transplant, and then tapered over the next couple of months to zero. And you can see in this case it was tapered to zero over the first few months.

She has been on no immunosuppression since that time, and her creatinine has been normal, and her biopsies have been normal. And she is now well over one year. Here is a typical biopsy at 60 days, normal kidney. No evidence either of chronic rejection, which seems to be another advantage of tolerance over standard immunosuppression. This is the patient in the middle. She just recently got married. That is her fiance at that time. So it does work, it is in the clinic, tolerance is a way of prolonging a transplant indefinitely, without the complications that you get from continuous immunosuppression, and without the insidious course of chronic rejection, which plagues all transplant patients in the clinic today.

Now our overall plan of attack in the Transplantation Biology Research Center and in collaboration with the transplant unit at Mass. General, has been to go from allogeneic models, and as you see we've gotten to human-to-human here, through concordant, and I should say that this same procedure does work for rat-to-mouse and baboon-to-cynomologous monkey for long-term tolerance for organ. But then to take it to discordant xenogeneic models, of course the ultimate goal to take it to the pig-to-human transplant.

Now most of the work in the pig-to-mouse model has been carried out in Dr. Megan Sykes's laboratory, and has led to many of the innovations that have now been carried out in the pig-to-baboon model by Dr. Kazuhiko Yamada. So the first initial transplants attempting to reduce this kind of tolerance were done by the same kind of mixed chimerism protocol I just showed you. Again, the elements are a low dose of irradiation, T-cell depletion, treatment with bone marrow at the same time as a kidney transplant. And in this case, extracorporeal immunoadsorption was necessary to get rid of the anti-Gal antibodies. We also used hDAF pigs, as Dr. Cooper showed you for the heart. We used those for these kidneys as well. But then the major difference between this procedure and the procedure for allo is the return of the natural antibodies, these anti-Gal antibodies. And when Dr. Cooper first joined our team over—now over six years ago, he attempted to prevent that antibody from returning by an enormous number of treatments, including a large number of drugs. And he has gone through some of that with you, and suffice it to say none of those methods prevented the return of natural antibody to anti-Gal.

They prevented the occurrence, the induction of new antibody. They prevented the—they were capable of preventing a lot of other responses, but not the return of these natural antibodies. And we knew right from the beginning, that when those antibodies returned, coincident with the return of anti-Gal, we started to lose our kidneys.

This is a xenograft, one of the early ones with the mixed chimerism protocol, which was doing beautifully until day 12, when we started seeing microhematuria. And you see there is a little bit of, when we biopsied, a little bit of hemorrhage in the kidney, which progressed to a full-blown antibody mediated rejection.

Now the other means that we've approached for tolerance, which does not involve mixed chimerism, and the reason being that we have been unable to get long-term chimerism in the pig-to-baboon like we get in the allo, has been another means of inducing tolerance at the T-cell level involving thymic transplantation. This, too, started in the

mouse with studies from Dr. Sykes's laboratory on tolerance across a discordant barrier of pig-to-mouse, and the method is really quite novel. What it involves is putting a little tiny piece of neonatal pig thymus from one of our inbred strains of pig under the kidney capsule of a mouse. Now this mouse was treated by that same kind of protocol we use for the mixed chimerism approach, which involves T-cell depletion, and in this case also thymectomy. So the only source of new T-cells in this mouse now is from that pig thymus, because it has been T-cell depleted and thymectomized. The tiny little pieces of thymus that were put under this capsule, 17 weeks later looked like this (indicating). They've grown into what looks like a normal thymus. And then histologically you see beautiful thymic tissue. Here is the kidney, and there is the thymus. So it becomes a thymus under the kidney capsule, and most importantly, it functions as a thymus. This is a normal mouse thymus, and this is a thymus that is in the pig stroma, and you see if you look to the mouse T-cells, this is the anti-mouse CD4 against the anti-mouse CD8. They are identical. They have the same kinds of T-cells growing up, mouse T-cells, but being educated and produced in a pig thymus. That is true of this slide as well for other markers.

So this allows, thymopoiesis, T-cell formation, most importantly from the point of view of transplantation, when we put skin grafts on from the pig, in this case it was the father of the litter, now we have inbred pigs, so we can use totally inbred. We can use the skin from another inbred animal, but you see that skin is accepted, and it's accepted long-term, and specifically.
So on that basis, we have now attempted to use thymal organs—thymic transplants as a means of inducing tolerance against this discordant barrier.

The initial work, again, done by Dr. Yamada, was done in an allo model, where he took a juvenile pig, took pieces of thymus from that pig, and implanted it under the animal's own kidney capsule. You see it right here (indicating). Now the reason for doing this is that unlike the mouse, where can just put little pieces of fetal thymus under the kidney capsule, and it grows into a nice thymus, that doesn't happen in large animals. In large animals, those little pieces get rejected. They get rejected during the time it takes to get vascularized.

So what Dr. Yamada did is to use autologous thymus, autologous kidney capsule, so that even though it goes through a process of revascularization, because it is autologous, it can't get rejected. So what happens is after two to three months, we take out what is now a thymokidney. Here is the kidney, here is the thymus. Here is the—it is right under the capsule, and that thymokidney we have found, and it has been published by Yamada, is capable of inducing tolerance across a full MHC barrier in the pig, from one pig to another. So this is another way we thought we could induce tolerance, perhaps across a xenogeneic barrier.
Now here is one of the xenogeneic kidneys. This happens to be from an hDAF animal. You see here day zero, you see after two to three months of residence in the pig, you have nice thymus underneath the kidney capsule. Here is what it looks like histologically. Here is kidney. Here is thymus, beautiful, normal thymus.

And now Dr. Yamada also has developed a means for transplanting the thymus as an organ itself without the kidney in those cases where you might want a thymus transplant plus some other organ, and this involves a direct anastomosis of vessels from the pig thymus into the abdomen of the baboon.

So by those two means, he has developed ways of transplanting the thymus of a pig to a baboon. We have the thymokidney, which is really a very easy operation, we have the vascularized thymus, which is much more difficult technically, but also he has now got working.
So what happens when we do this? This is now a protocol of xenotransplantation of the thymokidney, or the vascularized thymus plus the kidney into a baboon. Now, the immunosuppression we start off with is pretty similar to what you saw Dr. Cooper start off with, with a couple of additions, one is that we thymectomize these animals, because we are going to be giving them now the pig thymus. And in the initial study, we used all the other elements, which was multiple adsorptions, extracorporeal adsorptions of anti-Gal, cobra venom factor to block complement, anti-CD40 ligand, or anti-CD154 to try to prevent antibody return, and then gave a thymokidney, or a vascularized thymic lobe plus kidney as a transplant to these animals.

Now what did we find? Well, here is one of those thymokidneys from an hDAF pig. We also used, and again, to try to avoid the natural antibody problem, we used the hDAF pig, as well as all of these other manipulations. Here is a thymokidney on day 15, a normal, beautiful, functioning kidney in that baboon. But again, by day 27, we started to have some hematuria. We looked at it, and the biopsy shows the appearance of an incipient vascular rejection coincident with the return of anti-Gal antibodies. Some of these animals even had developed what looked like T-cell

hypo- or nonresponsiveness specifically to their donors. This is an anti-baboon response and a mixed lymphocyte reaction. The blue is anti-baboon, and the red is anti-pig. And you see that pretransplant it has both—this baboon has both an anti-allogeneic baboon and anti-pig, but after the transplant, at day 27, it specifically has lost the response to pig, and so has this one. This one in which we actually lost the kidney, took it out, remained hyporesponsive specifically to pig all the way up to day 60. Without the thymus. It eventually lost that hyporesponsiveness as well.

So in conclusion, of those initial studies, we have shown that thymic transplantation may have tolerized at the T-cell level, as may have mixed chimerism, with standard immunosuppression, did not prevent return of natural antibodies and thymic transplantation protocol did not prevent the return of these natural antibodies. That is why we needed the Gal knockout pig.
Now the Gal knockout pig was made, as Dr. Cooper told you, in collaboration with our colleagues at Immerge, and their colleagues, and involved a knockout of alpha-1,3-galactosyl transferase, the same enzyme that is missing in humans and in all Old World primates. It is missing in us as an accident of evolution, but it is now missing in these pigs, and therefore even though we have plenty of anti-Gal in our baboons, or in us, the pig no longer has Gal on its cell surface. These were the first Gal knockout heterozygous animals which were reported in *Science*, 2002. The heterozygotes have now been bred to homozygotes. This is the first homozygous Gal knockout animal, actually produced by a second knockout, in this case where we now have homozygous breeding from the heterozygotes.

So what have we changed in the tolerance regimen? We got rid of the multiple extracorporeal immunoadsorptions that were needed to get rid of anti-Gal, we got rid of the treatment with soluble Gal sugars, as Dr. Cooper told you about, we got rid of the cobra venom factor needed to block complement, but otherwise the procedure remained the same.

A treatment with T-cell depletion, MMF, steroids in most of these animals, I'll show you one animal Dr. Cooper already referred to with no steroids as well, and then we have a thymokidney, or vascularized thymic lobe plus kidney, and the anti-CD40 ligant antibody.

Well, the first—these animals now, we have done 11 such transplants. The first such animal, or one of the first such animals is baboon 113, unfortunately, this animal died during the replacement of an infected extravenous line, and this is what its biopsy looked like at postoperative day 29, essentially a normal looking kidney, and an absolutely normal histology. And when it—when it died on day 68, we were obviously very unhappy about the loss of this animal during the anesthesia to replace the line, but were amazed when we got to the operating room to find a normal kidney. That is the first time I'd ever seen a normal kidney on day 68 in a xenotransplant. Now these are functioning normal kidneys. These kidneys are supporting the life of the animal. This is not an accessory kidney, this is a life-supporting organ, with—I'll show you the creatinine, et cetera, in a moment. In addition, in this animal, you can see the thymus was again becoming to repopulate with thymocytes, which I think is the sine qua non for the actual induction of tolerance, although the animal was still on immunosuppression at this point, and was still being tapered.

And this is now one of the thymokidney transplants, where we do the thymokidney rather than the vascularized lower lobe plus thymus. This particular animal is the one that was done most recently, with a steroid-free regimen, no steroids. This animal was on the—was T-cell depleted with anti-CD2 antibody and antithymocyte globulin additionally, and then it was treated with the anti-CD154 monoclonal and MMF, and that is all. It did have cobra venom factor over the first week, just as a preventive measure, and then stopped. And this animal, here is its creatinine curve. This creatinine was normal. This was a life-supporting kidney out to 74 days, when it had a small bump, which was treated with FK506, and potentially because of rejection, although we don't really know if it was, it appeared to respond, and was back down to essentially normal, and it died unfortunately on day 83 of a—of what appears to be a myocardial infarction. We are not sure of the reason why. It had nothing to do, that we can tell, from the renal function. And the kidney on day 60, here is a biopsy, normal kidney, and absolutely normal histology, functioning kidney with normal creatinine on day 60, through to day 83, so it is really a remarkable change, a quantum jump in what we are obtaining, by now using the Gal knockout, when we use this tolerance induction regimen.

Now here, in addition, is some evidence in that particular animal that the animal does look at specific hyporesponsiveness, at least. This is a killing cytotoxic key lymphocyte assay on that same animal day 78. You see

here is the ability of the cells from that animal to kill the cells of the allogeneic baboon, and here you see the anti-baboon response, and here is a supplemented IL15 [*phonetic*] way of increasing the killing you see. So against an allo target, this animal recovered its immune responsiveness. But against a—against that same animal, against the donor, the DE pig type donor, with or without IL15 added, it has no response. Here is a naive baboon in blue showing the typical response, showing the typical response without IL15, so it looks like it lost its response in the assay against the pig. Here is a summary, then, of all the Gal knockout kidney survivals versus what we had previously with the hDAF kidneys, and you can see that there is an enormous jump in the survival times with the Gal knockout.

In addition, the kidneys look qualitatively different. They look normal when they're biopsied. Now we've done only two animals with a standard immunosuppression of the kidney. Most of the standard immunosuppression studies all were done in the heart model that Dr. Cooper presented. Both of these have rejected at about the same time that we used to see with the hDAF, or the normal swine kidneys within the first month or so. When they do reject, they have typical evidence of rejection, including the microthrombi that Dr. Cooper showed you, including T-cells infiltrating in this particular case, and evidence for induction of new antibodies, although not that much antibody, but nevertheless, it results in a biopsy that looks like they were antibody mediated.

So in summary, the renal transplants from the first available Gal-T knockout pigs showed that they do not undergo hyperacute rejection without requiring any attention to anti-Gal antibody, natural antibody for complement, they therefore did not require any adsorptions or inhibition of complement. Kidney alone without the tolerance regimen still rejected on day 33 and 35 with the kidney, with the thymus, either vascularized thymus or the thymokidney has now gone from maximum survival of 30 days to over 80 days—to 83 days.

So again my acknowledgments to a large number of people who are responsible for these advances, and that is where we now stand on the tolerance induction protocol for renal transplantation in pig-to-baboon.

DR. GROESCH: Thank you very much, Dr. Sachs. We do have—we are running a bit behind, but we do have time for some questions and a brief discussion.

DR. ALLAN: Yeah, that was beautiful, beautiful work, absolutely informative work. Question on the microthrombi. Maybe David or you could answer this. Have you looked at like IgM responses to pig antigens, or IgG responses to pig antigen, to see if microthrombi are due to de novo anti-pig antibodies.

DR. SACHS: That is a good question. We don't know the basis of that yet. It could be—we think it could be antibody at a level below what we could easily detect, antibody to other determinants. It could be some other problem with coagulation cascade. as Dr. Cooper mentioned, in one animal that was treated with aspirin, he didn't see it, of course, as only one animal. It is an area that we are still actively investigating. We don't know. I think that there are many causes of that kind of a problem, and I think it is seen in allogeneic transplants as well. And I think there are also many potential remedies to be looked at.

DR. SWINDLE: Yeah, just for technical clarification, when you are doing the original surgery, you are doing a bilateral nephrectomy on the baboon, and then reimplanting the kidney in the ileum? Is that the—

DR. SACHS: That is correct, although there are, in some cases we have been, rather than doing bilateral, nephrectomy, we do a unilateral nephrectomy, and a ligation and division of the ureter, which is something that has been devised by Dr. Ben Kozume [*phonetic*] for it allogeneic union problem. It allows the baboon to remain alive after you, if you have infection, after removing the xenografted kidney. Otherwise it's a total nephrectomy.

DR. SWINDLE: The creatinine levels you were showing were strictly with the pig kidney in the baboon.

DR. SACHS: Yeah, absolutely. The one animal I showed in the beginning, in the pig stat study, I showed you at day 60 MLR, even though the animal had lost its kidney back at day 29.

DR. SWINDLE: Very impressive work.

DR. KASLOW: Very nice, David. Have you looked at the thymus with regard to expression or cellular profile, and it looks like they are not getting infection, but do we know anything about their ability to respond to an array of antigens?

DR. SACHS: The only one we've seen so far is the one I showed you, which developed a CTL activity against the allo baboon. These animals, remember, are still being tapered from immunosuppression. They are not off of immunosuppression yet.

DR. SYKES: David, just to clarify John Allan's question, I think he was asking whether there is any anti-pig antibody detectable in these animals.

DR. SACHS: In the animals who have had this protocol, we have not been able to detect any anti-pig antibodies coming up. In the animals who have developed—in the two animals that were treated by the standard immunosuppression, where we did see the appearance of microthrombi, we did see antibody against non-Gal antigens, and it could correlate with those thrombi.

DR. VANDERPOOL: Dr. Sachs, what do you see in the future as ways to increase the life of these recipients?

DR. SACHS: Well, I think it is really—it is a point right now, it is—we only had these Gal knockout pigs for a year now, and we've only had a total of I think eight pigs that we had, Gal knockout pigs to use, so there have been a maximum of eight hearts, and there have been 11 tolerance kidneys and three control kidneys is approximately that is what we have been able to do, so we are just at the beginning of this, but I have never seen these kinds of histology in a functioning kidney before. So I think the problems we are now seeing, even where the animals have been lost, they were lost in those long-term cases, in one case with infection, the animal was still on immunosuppression, and with an infection, another one was an anesthesia death, but again, during a replacement of an infected catheter. So again there was an infectious problem.

Then the last one from a heart attack, a myocardial infarction, a small one that probably led to an arrhythmia, and it could be just something we'll never see again, or it could be something we have to go after. We don't know the basis of it. We are concerned that perhaps it is a complication of anti-CD154, which has been known to cause thromboembolic phenomenon in clinical studies, and in some—and in monkeys, and which might have to be replaced by a different reagent. But I think that all of these kinds of problems look to me to be addressable. I don't see anything yet that looks to me to be something that would stop us—would stop the progress, I mean, from the point of view of science, and indeed it is a time at which I'd like to see us really thrust forward on the science side. It is another change. You know, these things go in quantum changes, and we reach plateaus of survival. And they go like this, and this is a new one that has just come up.

Now unfortunately, I guess this is something not a topic for discussion, the funding situation for this science has dramatically changed, too, because Novartis has pulled out as a sponsor of what used to be the major sponsor of research in this field, and has made a corporate decision no longer to do so. They pulled their support from Immerge. Immerge therefore had to pull back its support from the work that we're doing, so there is a problem in how quickly we'll be able to move forward. But I think it is a long time. It is the right time, right now, to push forward vigorously. We are finally starting to see scientific advancement at this level.

DR. VANDERPOOL: Final question, is if my memory serves me correct, some years ago on the FDA Xenotransplant Committee, we talked about—the committee almost took a consensus vote, and by a majority agreed that if one could see therapeutic success for an average of 60 days, one should move to human trials. I'm not saying that is the standard we are going to follow, but could you see that as a possibility in the not so distant future?

DR. SACHS: Well, I certainly see that as a light at the end of the tunnel that I now see quite clearly that is where we are going. We are driven on in this work because patients are dying every day from lack of organs. And every time that happens, one thinks this is where we need to go, but we don't want to do it before it is time. And I think it's certainly, this looks like progress towards that goal, but I don't think we are quite there yet.

DR. SYKES: I'd like to just add my two cents worth in response to your question about, the direction. I'd like to point out that that last kidney that you saw where the animal died with an intact, pristine kidney on day 83 was an

animal that received less immunosuppression than any other animal, and that that animal received no steroids at all, and that the tolerance—the goal of the tolerance protocol is to get the animal completely off immunosuppression. I think that the exciting direction for this work is less immunosuppression, and hopefully less complications.

DR. SACHS: I think even if we have to look at immunosuppression, as Dr. Cooper showed, immunosuppression is manageable, and certainly in that animal, as Dr. Sykes points out, it was only on MMF, and had a reasonable dose, and was being tapered from everything else at the time that it died from this infarct. So I think the—so I think it is certainly getting to that range, but I think we need to see more animals going that long or longer, and we need to correct some of the other problems that we see that have lost—that have led to a loss of our animals. I mean there are many people who say that it would be a lot easier to do this in human beings, because the care of the patient is so much easier in a hospital situation with the right kinds of monitoring. But I think, as you have seen, we have done pretty well in the baboons. It does take a very big effort, and I do think we can do even better.

DR. VANDERPOOL: Thank you very much. We are going to take a lunch break, and actually not a break to go eat lunch somewhere else, but to grab your lunch, maybe hit the restroom, or check out quickly, or whatever, and let's come back here in 15 minutes to continue eating our lunches and move into the next two presentations. Thank you.

<LUNCH BREAK>

DR. VANDERPOOL: Let us proceed with the next two sets of presentations as we complete our lunches. The first are updates on Porcine Endogenous Retrovirus by Dr. Dan Salomon, Dr. Megan Sykes, and Dr. Clive Patience, and that will be followed by an overview of the clinical study in Mexico City, after a brief discussion. And then finally, the immunological findings. We will proceed with these before we go to the second report of the subcommittee by the SACX.

DR. GROESCH: Okay. Thank you. Our first speaker is Dan Salomon, and he will be talking just about an update on PERV and xenogeneic infections. Thank you, Dan.

Agenda Item: Updates on Porcine Endogenous Retrovirus (PERV)

DR. SALOMON: Thank you, Mary, thank you, Mr. Chairman and ladies and gentlemen.

What I've decided to do today is basically review the last year of the literature and end with a brief discussion of some of the work that's going on in our laboratories, with the idea of just—basically this is a federal advisory committee. There's clearly been a series of ongoing discussions on xenogeneic infection risks, and it's always good to base those sorts of discussions on facts. And so it's always good at one point to just kind of take a look at what's happened in the last year in a critical fashion.

So this is a paper here that appeared here in the *Journal of Clinical Virology*, it's from the group at the Robert Culk Institute in Berlin, entitled "Porcine Endogenous Retroviruses: No Infections in Patients Treated With a Bioreactor Based on Porcine Liver Cells."

So to make a long story short, essentially a major medical problem worldwide is acute liver failure. This can occur as part of chronic liver disease in its end stage, but also can occur through pulmonate hepatic infections and toxic exposures. Unfortunately, besides from transplantation, there's not a whole lot of therapeutic modalities currently available for this. One possibility, therefore, that's been recognized for many years is the idea of essentially dialysis for the liver, which are these bio artificial devices in which they can be loaded with various sorts of cells; in the case of xenotransplantation, the interest, of course, has been in putting pig hepatocytes in these devices. And in that case there is a barrier between the pig hepatocytes and the device and the human blood flowing through it, and that barrier is semi-permeable, and therefore the issue can be raised, is there a risk for infection.

So this paper on a simple level joins three others that I know of published over the last three years that at least in these short-term exposures—these are four-, five-hour exposures to these devices—that there is no evidence for transmission of any pig infections, but certainly in this case Porcine Endogenous Retrovirus. Okay. Fine. Another negative study.

I think the other way to look at this in a more positive, broader way is that again, what's been going on in the background here is that scientists are developing assays that are suitable for clinical laboratory applications that can be used with human serum samples in really real-life situations to monitor these infectious risks. And they're validating these assays, they're developing unique antibodies, recombinant antigen-based ELISA assays are both described in this paper, and I think that's an important maturation, at least, of the clinical monitoring side of the field.

This is a review written by Dr. Meng from the Virginia Polytechnic Institute and State University, and it takes us in a little bit of a different direction. This just came out, this Current Topics in Microbiology and Immunology; it was edited by myself and Carolyn Wilson of the FDA, so I have to—I don't think I'm getting any money for this, but let me just say that I was involved in this in some way. But this is a beautiful review by Dr. Meng on Swine Hepatitis E Virus Cross Species Infection and Risk in Xenotransplantation.

And so what he's identifying here, again, to make a long story short, is the identity of a swine hepatitis E virus. It's clear now that two important things in the context of xenotransplantation; number one, that this virus is known to be xenotropic, in other words, it can certainly affect both non-human primates and humans, and that it is present in U.S. swine herds in maybe 80 to 100 percent of individuals in various studies. So this is definitely around, and therefore—and it's xenotropic, and therefore it is a very important possible pathogen.

What I thought was extremely interesting, and it educated me—some of the infectious disease experts in the group already knew it—but was this last point that there's now evidence that a number of new strains of human E virus that have been observed in several countries, that when they've gone back and looked at the swine E virus in the local areas, it turns out that there is at least molecular evidence that there was—that the reservoir was the pigs in the local areas, and that it was infecting and adapting and becoming human hepatitis E virus.

So I think that this is yet another example that I think, to put into proper context, has been well documented, that animal populations, both wild populations and domesticated populations, are reservoirs for pathogens, and those pathogens move routinely into human patients. Or I guess they move into humans, and then the humans become patients. I think that's another important distinction.

This is a paper that just came out from the Mayo Clinic group, where they did a study where they essentially transplanted human hematopoietic stem cells into pigs, which is sort of the inverse experiment, I guess, to what we might possibly do in a clinical situation, which was to transplant pig hematopoietic stem cells into humans. And what they observed was that at a very low frequency, and this is very important not to be—to raise any major alarms here, but at a low frequency, there was fusion between the pig—between the human hematopoietic stem cells and pig somatic cells. It's not quite clear from this paper where the fusions occurred, but they seem to have occurred in multiple compartments.

Is that novel? No, it's not novel. We've known now for at least almost two years that with human hematopoietic stem cells, as well as other hematopoietic stem cells, that this sort of cell fusion with somatic cells in a recipient animal is known. And the reason that all came out was because there was this whole set of claims for bone marrow derived hematopoietic stem cells giving rise to neural cells, hearT-cells, muscle cells, et cetera.

What is now clear is that at least a significant portion of those claims could be re interpreted by somatic cell fusion. So the CD34 hematopoietic stem cells didn't necessarily become brain cells or hearT-cells, they fused with brain cells and hearT-cells, and so they fluoresced with the green fluorescent marker protein. And this is the way science moves on. It wasn't bad research on one hand; it was wonderful research to better understand this idea of somatic fusion.

Anyway, when somatic infusion occurs, whatever is there is going to get fused. And what they found here, amazingly, is that if you got fusion, and the pig PERV proviral DNA was part of the fusion, that you then made PERV in the fused cells. And that was fine. So this is very interesting, very appropriate, very consistent with what's been going on. The only thing that bothers me is by the time it got into the late press, it was that you're walking down the street and you get bitten by an animal, and that infuses CD34 stem cells in you, which then fuse,

and you get a novel pathogen. And I'm afraid that somewhere a long the line they lost me on that one. So don't worry, you can walk down the street without fears of this type of transmission.

This is an interesting one, "Transplanting Encephalomyocarditis Virus-Infected Porcine IsleT-cells Re verses Diabetes but Transmits the Virus." And this is from the University of Minnesota Group in Xenotransplantation this year. Basically, the porcine encephalomyocarditis virus is a bad actor. It's known to infect humans, it's known to be xenotropic, it's known to infect mice. And when it infects mice, it kills mice.

And so they transplanted pig islets from pigs infected with this virus, and the pig islets worked for about five days before the virus killed the mice. I have an internal dialogue about this one. The first part of me said, this is a good example of where an IACUC might not have subjected these mice to this study because you sort of know the result; and then the other part of me said, yeah, but if I didn't have this paper in press in a peer-reviewed journal, and I just stood up and said, this is a bad thing, then Bill might accuse me of doing hand-waving and raising unnecessary fears.

So there is something about this dialogue in the peer-reviewed press—and I am very serious about that. This is the dialogue I had, and I decided to share both comments with you because I think they're both true. The conclusion here is this is not a good thing to do.

This is another paper that is very important, in that monitoring for potentially xenozoonotic viruses in New Zealand pigs comes—and it was published in the *Journal of Medical Virology* this year, it comes from the laboratory group of Diatrans in New Zealand. This is a very nicely done study. They do monitor, by the way, I want you to notice, for the pig encephalomyocarditis virus, so that's a good one, chalk that one up to them, and also for the hepatitis E virus. So this is very a propos, very appropriate, and they point out that they've developed good technologies for doing this in the laboratory—another positive—and it's now possible to apply these to protocols for screening donor herds for xeno. That's a good thing.

And in the end they say that this allows selection of a possible source for transplantation. My only concern here is that this is a company that claimed that they had a source herd about two years ago and were moving forward with xenotransplantation; however, to be fair, it's possible that all this work had been done two years ago and they were just publishing it now.

But to put this into a bigger context, this is another review article from our book, and this is written by Prin Paul and his group from the University of Nebraska, and he reviews exogenous porcine viruses, including all the ones that were in the previous paper, but then a whole list of others. And I think just the point here is to say that one of the whole objectives, I think, of the field is to realize there are potential risks, not to blow them out of proportion, to see them in the context of the fact that there are these viruses in all domesticated herds such as pigs, that there are scientists that are capable of devising assays, and are doing so actively, and they need the support of all of us to develop those sorts of assays and implement them.

So all this stuff is moving forward, and I think that's part of a re assuring development and maturation of the field, and we all just ought to realize that that's going to be a moving target, and that in moving forward to clinical trials, we just want to get everybody together and get the best thing going possible, and then go forward realizing you can't cover all the bases all the time.

This is a study from Carolyn Wilson and Takala Argo and their group at the CBER at FDA that was just published in the *Journal of General Virology*, essentially exploring the guinea pig as a model for Porcine Endogenous Retrovirus infections. And a very fine scientific study, the bottom line being that the guinea pig did not demonstrate—it demonstrated one round of infection but didn't develop productive infection. That's been a problem with the small animal models; it's the same thing as we found with allo G skid mice. And it just suggests that there are other barriers to developing productive infection, at least in these small animals, so we need more work to develop better models.

I think this is a really nice piece of science. This is a group from the Arasmus Medical Center in the Netherlands, published in the *Journal of Virology* just at the end of last year. And what they did was they expressed a single-domain antibody against a molecule called P-15 matrix protein, which is part of the viral Gag, and they expressed

it—in this case, it's just a model. They expressed it transiently in cells that were producing porcine endogenous retrovirus.

And what this construct does, think of it, if you will, maybe as a form of gene therapy or as a model of something that could be done transgenically. What it does is in the endosome, it essentially coexists with the Gag, grabs it out of the endosome, and essentially takes it out of circulation so you can't package infectious virions, and essentially prevents the cell from being infectious for PERV.

And I think this is a beautiful piece of basic science on taking advantage of what we've learned about porcine endogenous retrovirus sequence and function. It's also very consistent with work that's been done with other Gam retroviruses.

So what's the bottom line? Well, one of the things you could do is engineer such a construct into a transgenic pig, and I'm not trying to say again that the risk would be zero, but you would again dramatically reduce the possibility of packaging infectious virus. And everything you do at that level incrementally reduces the overall risks of xenotransplantation, and I think that's very positive. So I thought this was really one of the more exciting papers, from my point of view, of last year.

So I would like to finish by just reviewing very briefly what we're doing in the Scripps PERV consortium. This consists of the people in my laboratory, the FDA, led by Carolyn Wilson and her group, and at Immerge led by Clive Patience. And I just want to point out that this to me is a model for this sort of collaboration in which we have represented academia, federal—a basic science lab in a federal regulatory environment, obviously, and a biotech company, and it's been a really wonderful collaboration.

So essentially I want to tell you about three possible things that we're working on right now that kind of chart a direction forward in this area. The first is that there's the viral entry defect in non- human primate cells. So two years ago we published, in the *Journal of Virology*, data that showed that non-human primate cells again could get infected by porcine endogenous retrovirus, but did not—now, these are cell lines in culture. Did not demonstrate the kinds of productive spreading infections that we found in many different human cell lines and culture.

And this raised two major issues for us in this first paper. The first was how well can you then use non-human primates, because we studied baboon cells, Rhesus, Macaca, and African green monkeys, so a number of different primates. So if there's some sort of block to viral entry in these non-human primate cells, how does one interpret the negative data that's been published in so many non-human primate study in xenotransplantation.

The second interesting point, and the way we want to go forward in the future, is what is a better model. In other words, are these non-human primate cells more like human primary cells? Because if you remember, all the existing data for human studies of PERV pig contacts, transmission of PERV, have all been negative. So is it possible that the—what the real problem has been is that the human cell lines, because these are immortalized tumorigenic human cell lines, that for some reason they are not the representative model, and that actually the non-human primate cell lines are telling us that human primary cells have natural blocks to PERV infection.

If that were indeed true, the risk of PERV infection would be dramatically less than we currently think, and it might even be nonexistent. And again, my point is, is that this should be a question not answered by dramatics, but rather just by good old-fashioned good science.

So the hypothesis is that there was a viral entry defect, and Carolyn and her group, of course in collaboration with the whole consortium, has actually found a cofactor for viral entry and cloned it, and we're currently investigating its possible role as explaining this viral entry defect. Clearly, once you identify a molecule that's a viral entry defect, we can quickly test whether these things are present in human tissues and answer the question.

We also think there's a viral assembly defect. So this isn't projecting very well, so let me just explain what we did. We took non-human primate cells and we said, okay, fine, we've demonstrated that there was a viral entry defect, but is that the only defect? So what you basically do is you bypass viral entry by pseudotyping the virus, so you essentially take the whole viral entry defect out of the equation. So we created stable non-human primate cell lines

that had a ton of PERV in them. Now, it wasn't naturally occurring, we pseudotyped it, but that was the whole purpose. Okay?

And now we took these cells and we said, now that you've got past the viral entry defect, everything is normal or not. And it turned out that nothing was normal. And so there was a second defect in these non-human primate cells, that the end effect was that they didn't assemble virus properly.

And this is really interesting, because from a scientific point of view, it led us to this hypothesis: The failure to observe productive PERV viral infection in non-human primate cells, despite the presence of PERV RNA and PERV proteins in these cells—and I didn't show you the data, but you have to trust me on that—is due to a defect in PERV viral assembly, and that mechanism involves the traffic of Gag. Remember the Gag protein I was talking about with the lama virus?

Now, again, I don't have the time to describe this, but this is a picture taken from a review a couple of months ago, and this is on HIV budding. And again, let me just summarize what I did yesterday; I was at the NCI at Fort Detrich, and we spent the whole day and a meeting on viral budding and assembly mechanisms. What's really fascinating to me is we started working on PERV five or so years ago at a time where it was, like, oh, this is a virus, who cares about it; I mean, all these people are dying of AIDS, let's concentrate on that. And that's all appropriate. What's fascinating is, is just staying on the scientific track of PERV, it's taken us full circle to where we're thinking about the same problems all the HIV guys are thinking about. And that, again, I think underlines the fact that if you just do good science, it takes you in the right direction.

So we now have evidence that there are defects in Gag traffic, and these are these just incredible mechanisms. I really wish I had more time to make this come alive for you, because to me this is one of the most interesting areas in retrovirology right now. But suffice it to say that there's a whole lot of things going on that takes these Gag proteins to the membrane where you can assemble a virus, and we're going to try and work through these molecular pathways to determine the defect.

And if we find the defect, again, we now have the viral entry defect, we have a viral assembly defect, and we can go back and look at human tissues and determine whether these defects are also present in human tissues. If they are, then again, the risk that we think of as PERV may be significantly less or non-existent. And that's sort of the direction of the research.

And the last area I want to tell you about is Clive Patience at Immerge and I had the great honor to participate on that also with Broadman Weiss and others, where Clive essentially cloned the human receptors for PERV. This was a major step forward in developing a new animal model, because what we realized in the process was that the reason the mouse and probably the guinea pig isn't such a great model is that the receptors on the mouse and the guinea pig cells are not good.

So now that we have the cloned human receptors, the obvious suggestion was to put that and make a transgenic mouse. So that's taken us about a year, and again I thank the NIAID for NIH funding for this project, and we have just now got the founder animals going. And these are the first experiments I wanted to share with you.

What you're looking at here is 293 T-cells, which is a human cell line that is easily infected with PERV. I mean, any post-op that comes in the lab, that's their first thing to do. If they can't get this one infected with PERV, they're fired. And so basically here you see the level of infection of PERV, so that's your positive control.

We know kidney—I made a primary kidney line from the transgenic receptor positive animals, and they are infected in vitro to the same extent as the 293 control. We took splenocytes, fresh splenocytes, from these receptor transgenic animals, activated the PhA and exposed them, and you can see that they're actually almost two logfold greater infected than in the 293 control, which was really remarkable. And this is the control of the same splenocytes activated in the same PhA, but from receptor transgenic negatives. And these actually were litter mates of these.

So this is just the beginnings. I had another slide which I took out because it's so preliminary, but we've also begun experiments where we're injecting PERV virus directly in to the animals, and in the first experiment done at three-week time point, we have multiple tissue compartments infected with PERV.

So what I'm hoping to do in the next year with my collaborative group is really characterize this model, where we may truly have a small animal model that is infectible by PERV and produces productive infection. I can't say that we have that yet. I don't want to misstate the facts, but that's what we're going to do for the next year. And if we can, I think that will be another step in advancing things forward.

So with that said, I wanted to go back to my last slide, my second slide. This breaks every rule of good slide-making, and I know that. But I wanted to say this, particularly as I'm going off the committee. So my final kind of comments here are in the case of xenotransplantation, we're trying to establish the risks of both known and unknown viruses causing diseases that we've never encountered and may not even exist. Okay? So Bill, you and I aren't really that far apart.

As a scientific principle, establishing the significance of negative results is very difficult; as a principle of public policy, negative results without the requisite underlying knowledge base stresses the unknown, and as such, they're difficult to use as the basis for re assuring the public that their safety is assured at the cutting edge of biomedicine. And so Bill, if there's any comment I have to you from this morning's comments, is that we don't have to be panicking, we don't have to be alarmists, but we do have to do good research and found our decisions on good basic science.

Establishing the molecular mechanisms of xenogeneic infections in mammalian tissues, not just PERV but others, is our best response to these uncertainties in xenotransplantation. Moreover, and this is another really important point, I believe, is that xenogeneic infection risk is part of a larger concern for emerging infections moving from animal reservoirs to human populations. And I think we need to see this also in this larger picture.

And that defines the significance of this committee, and it is the importance—it's critically important to continue funding of this kind of work. Thank you.

DR. GROESCH: Thank you very much, Dan. Appreciate the overview and the description of the work. I think we're going to go right into the other two presentations and then we'll have a brief discussion period at the end.

So our next speaker is a SACX member, Dr. Megan Sykes, and she's going to be talking about approaches to xenograft tolerance, and a model for studying the potential of PERV from surviving porcine xenografts to infect human cells in vivo. Thank you.

DR. SYKES: Thank you, Mary. Well, David Sachs has really introduced this first slide in which I outline the two major approaches to tolerance induction to xenografts being studied in our section, in our center. One is mixed hematopoietic chimerism, and this is a very promising approach that even works in closely related xenogeneic species, but hasn't yet succeeded in the highly disparate pig to primate combinations because of limitations so far in our ability to really get good marrow engraftment because of various barriers.

And then the second approach you already heard about from David is the thymic grafting approach. We've actually obtained proof of principle, using an immunodeficient mouse model, that human T-cells can be tolerized to pig antigens in a porcine thymic graft.

And what I'm going to talk about today is —so what I'm going to talk about today is another mouse model in which we have been able to obtain long-term survival of relatively large amounts of pig and human cells, and actually being able to address the question of mixed chimerism of pig cells being able to tolerize human T-cells. And this is work from the laboratory of Yong-Guang Yang, who is a senior investigator in my section at the TBRC, and his postdoctoral fellow Ping Lan. And this work is in press in *Blood*.

And basically what Yong-Guang has done is generated transgenic immunodeficient mice that express porcine hematopoietic cytokines, IL-3, GM-CSF, and stem cell factor, and he's already shown that these mice can support very high levels of pig marrow engraftment and chimerism.

And so the question that we've now asked is if we put in a human thymus and hematopoietic stem cell graft, can we now generate human T-cells in a thymus and render them tolerant to porcine antigens by inducing porcine mixed chimerism. And the only condition these animals need is three grade of total body irradiation, a somewhat lethal dose.

And what is achieved is long-term survival, as you see here, with a pan-pig antigen on the X axis, of pig bone marrow drive cells in all the porcine organs, a long with human leukocytes. These are in fact T-cells in all the organs of these animals. And these cells down here are mouse cells, not stemming from the human or the pig cells. This is long-term. This is 25 weeks after transplantation. You have this coexistence of human T-cells and porcine hematopoietic cells in these mice.

And this is what one of those human thymus grafts looks like. It was placed into the kidney capsule; it was very small at the time of implantation, has grown very large, has normal structure of a normal thymus, and supports what looks like perfectly normal human thymopoiesis. And again, this is long-term, 20 weeks post-transplant.

And what we think is most important from the point of view of tolerance induction is that in these human thymus grafts, you not only see human Class II positive cells in the important place where they're needed to induce tolerance, you also see porcine Class II positive cells in that human thymus graft. These porcine cells have migrated spontaneously to the human thymus graft. And these cells, if you look at them closely, have a dendritic cell-like morphology and they express a high levels of pig Class II, and we think these are very important tolerance-inducing cells.

And indeed, the human T-cells developing in these human thymuses in the presence of porcine mixed chimerism are specifically tolerant to the porcine donor. These are using David Sachs' inbred miniature swine, where we have defined MHC antigens on our donor pig. And we can show that animals that just get the human thymus and human stem cells have T-cells that respond to the donor type of pig that that animal didn't get—third-party pig—and human alloantigens; whereas animals that got porcine mixed chimerism along with the human thymus grafts show specific unresponsiveness to the donor pig strain. They respond to other pigs and they respond to human alloantigens. So this is specific tolerance induced centrally in those human thymus grafts.

So to summarize, implantation of human fetal thymus and liver tissues leads to human thymopoiesis and functional T-cell development in these transgenic immunodeficient mice. In these mixed chimeras, human thymic grafts are populated spontaneously with porcine antigen presenting cells, and human T-cells developing in these mice are specifically tolerant to the porcine donor MHC.

Now, these human cells repopulating the mice are mainly T-cells coming from the human thymus grafts, and they respond to allogeneic and xenogeneic stimulators in MLR assays, as I showed you. But one limitation of this model is that those human T-cells don't seem to function sufficiently to cause rejection of skin grafts, for example. So we're really unable to study tolerance at the level of organs in grafting.

Recently, though, evidence in immunology has come out suggesting that the presence of antigen-presenting cells of the same origin as the thymic epithelium might play an important role in promoting the survival and optimal function of T-cells in the periphery. So what Drs. Lan and Yang did was ask the question, if they added additional human hematopoietic stems cells into the periphery of these mice to try and get development of human antigen-presenting cells, would they now have human T-cells that could reject pig skin grafts in vivo.

And so they basically used the same model I showed you, but now added selected hemapoietic stems cells, CD-34 positive fetal liver cells, from those human donors and gave them IV, in the hope of getting better peripheral reconstitution of non-T-cells of human origin.

And that's exactly what they got. The animals that got the human stem cells injected IV, with the white bars, had much higher levels of human cells, including T-cells, suggesting that human T-cells survive better or were generated more efficiently. But also now they see human B-cells at much higher levels, monocytes at higher levels in all the organs, and importantly, Lin-DR+CD11c+ cells, which are dendritic cells. So they're human dendritic cells in the periphery of these mice.

And this is an example of a lymph node from one of these mice, and this is really remarkable, to see normal lymph node size in an immunodeficient mouse. They normally have tiny, almost undetectable lymph nodes. And you can see that these lymph nodes contain human CD3+ cells, T-cells, and B-cells. And here's other stains. They also contain dendritic cells. The animals also generate human immunoglobulins, natural antibodies in the serum, both IgM and IgG, at much higher levels than animals that don't get the peripheral stem cells injected IV.

And most importantly, these animals now have the capacity to reject skin grafts. So these are animals that got just human thymus and fetal thymus and liver grafts with peripheral stem cells; they reject pig skin grafts. Whereas the animals that didn't get the peripheral stem cells, despite developing some T-cells in the periphery, they're incapable of rejecting pig skin grafts.

So now we have a model in which to ask the question, when we now add the pig bone marrow back and induce the mixed chimerism of porcine cells, can we induce human T-cell tolerance to porcine skin grafts. And the answer is yes.

So these are, down here at the bottom, animals that received, again, the human fetal thymus and liver, and just the peripheral stem cells without any porcine cells; they reject pig skin grafts, regardless of which type of pig it comes from. This animal kept its graft longer, but had a severe mononuclear cell infiltrate of its graft.

In contrast, animals that got the same human tissues but also got pig bone marrow cells leading to mixed chimerism, all accepted the skin from the donor matched pig but rejected third-party skin. And animals that just got pig bone marrow didn't reject either. So this demonstrates specific tolerance, again, to the donor pig, with rejection of third party's pig of T-cells, human T-cells, developing in human thymus grafts.

So in summary, co-transplantation of human thymus/liver and stem cells can re constitute a functional human immune system in immunodeficient pig site and transgenic mice. These results provide the first proof of principle that central T-cell tolerance, intrathymic human T-cell tolerance to porcine xenografts can be induced in the human thymus through mixed hematopoietic chimerism. In these mice, human and pigs coexist permanently in relative large numbers, and we've been very interested in this as a positive model in which to ask whether or not PERV can infect human cells in vivo when the human and the pig cells are coexisting together for many months in relatively large numbers.

And so this is something that we've recently looked at in collaboration with Clive Patience and J. Fishman and others, and in particular have asked the question, when we use a pig from a herd that is known to be a non-transmitter of PERV in vitro, unable to transmit to human cells in vitro, what happens in vivo when these cells coexist for a long time with human cells.

So I'm going to stop there, and that's sort of a lead-in to Clive Patience's talk. Thank you.

DR. GROESCH: Thank you very much, Megan. And Dr. Clive Patience from Immerge Biotherapeutics is going to talk about progress in PERV research. Thanks, Clive.

DR. PATIENCE: Well, firstly, thank you for the invitation to speak here. As Megan said, I'm going to touch on the results that we've generated recently in the murine models. I think essentially the bottom line message is that the complications of mouse models are certainly becoming better understood.

Very briefly, due to time restraints, I'm also on the committee, and the public will be interested, obviously, on the Gal knockout PERV infectivity studies, a recent piece of data looking at the nuclear transfer technologies which ultimately led to the Gal knockout animals, and a couple of other publications which I thought were prudent to bring to people's attention which are impressive and which I think will be useful for the scientific report.

So going back to the model which Megan described, as Megan stated, we wanted to address the potential infectivity of a non- transmitting pig; i.e., a pig which does not in vitro transmit PERV to human cells, and we wondered if that might be different once it got into an in vivo setting.

In that regard, we transplanted, as Megan described, pig bone marrow cells that do not produce PERV. That was co-transplanted with human fetal liver cells. And I'm really having to reduce this study to really bare bones here. Following about a six-month period in culture, the vast majority of the animals , we sorted human cells from the animals, and the human cells showed absolutely no signs of infection with PERV. Very re assuring. But we had used a non- transmitter animal, precisely the type of animal that you would presumably use for a clinical transplant.

The surprising result was that in a number of the animals using quantitative PCR, we actually did detect an increased PERV load, increased PERV DNA load, more than could be accounted for by chimerism. And in the typical analysis which is performed using this type of model, the only conclusion that we could come to was potentially that PERV had transmitted to human cells in the model. That was somewhat of a surprise.

In order to look more closely at the type of PERV which it transmitted, because we are faced with the possible conclusion that an otherwise cryptic locus of PERV had become activated in vivo, we co-cultured with the only human cell which really supports—cell line, I should say, which supports PERV replication 20 degree. The only virus that we detected in the cells was low levels of PERV-C, which again, the conclusion would be that PERV-C had transmitted to human cells.

The really quite important statement here is that PERV-C has no human tropism, so what was it doing in the cell? We looked more closely at the human 293 cells, and we found high levels of murine leukemia virus which we found was replication competent, and the particular type of MLV, Murine Leukemia Virus, that we found was the type which is capable of infecting both porcine and human cells.

So ultimately our conclusion in here is that pseudotyping presumably the MLV was contributing to the presence of the PERV-C that we saw, and the pseudotyping of PERV genomes by MLV can result in an exaggerated potential of PERV transmission. I think if I can paraphrase from Dan Salomon, one of his earlier slides, I think ultimately the use of the non-skid animal is not a good thing to do because it makes the interpretation of positive results almost impossible.

How does one get over that issue? Well, clearly with the potential impact of xenotropic MLV, the obvious move is to move towards animal lines, animal strains which lack replication-competent MLV. There's a number of these, a few, there's a number available, so a lot of the work that will be performed will be performed in that type of animal. The pseudotyping effect seems to require porcine cells to be present, so obviously the use of cell-free PERV infectivity studies should still be valid. And as Dan has alluded to already, I think the most prudent course of action is to concentrate studies on PERV-A—PERV-A has the greatest potential of infection for human cells—and to use receptor transgenic mice using one of the lines which lack xenotropic MLV. And that's where the studies are heading.

Moving swiftly on to the potential of Gal-knockout pigs, the question which has been raised is with the removal of the Gal sugar from the pigs, it makes the organ that much more acceptable by the non-human primate, and ultimately we hope human immune system. That sugar is also responsible potentially for the inactivation of PERV, and has been touted as a potential protective mechanism that may be bypassed. Therefore, by genetically modifying the animal to be more transplant friendly, you may have an increased infectious risk.

We addressed exactly that question using porcine cell lines to start off with, and it's projected a little bit small here, but this is particle neutralization up this axis, and across on this graph were increase in the concentration of natural antibodies. This is anti-Gal. As you can see here, the virus that's produced from the Gal-null animals, the Gal negative, the Gal-knockouts, shows little if any sensitivity to neutralization by natural antibody. In contrast, the virus released from wild-type cells, when you get up to approximately 5 to 10 micrograms per ml of natural antibody, is showing sensitivity.

What is also not projecting that well here is that this maximal level of neutralization that we see was always less than 100 percent. We could never completely neutralize even Gal positive virus, indicating that the actual mechanism is rather weak.

Similarly, this graft was produced with purified natural antibody, and this is a similar study showing very similar results, but this time using human serum. Again, incomplete inactivation, but clearly a differential between the Gal positive and Gal negative.

And then lastly, a third way to investigate the study was the production of replication competent virus following virus treatment. Here you can see a human cell, plus or minus natural antibody treatment. But really the critical line here is this white circle at the bottom, which is Gal-positive virus treated with high levels of natural antibody, and we were still—again, you can see that although it's delayed in kinetics, the virus came up again, again suggesting that natural antibody anti-PERV effects were pretty weak.

Looking at the miniature swine, inbred MHC inbred miniature swine, which is clearly what a lot of people were interested in, we found that all of the Gal-knockout animals retained a non- transmitting phenotype; i.e., they still did not produce any PERV which infected human cells. That was very encouraging, because there was potential during the re processing—re-programming, I should say, for cryptic loci to be activated, and that was not the case in any of the animals that we've looked at so far.

As with the majority of miniature swine, they do continue to produce a pig-tropic virus, the PERV-C which I mentioned earlier. And again, I just don't have the time to present the data, but in all of the transplants that were very nicely alluded to by Drs. Sachs and Cooper, we've seen absolutely no evidence of transmission of PERV to the primary recipients, despite the quite elongated exposure.

The third point I would like to touch on is the potential effects of nuclear transfer. For those of you that aren't familiar with nuclear transfer, essentially the bottom line is that pigs are ultimately produced from what equates to single donor nuclei. So the genetic material of all stages of the production of the Gal-knockout pig should look identical, with obviously the exception of the targeted gene, where you're targeting the Gal-axial transferees.

We questioned the assumption that the animals would be consistent, and used Southern blot analysis of the genomic DNA and probed for two families of PERV, the particularly important ones; the PERV-A, which is a human-tropic, and the PERV-C, which is the pig-tropic virus.

And this was the result which I'll very briefly take you through. As you can see here, going down the left-hand side here is a quick tracing of how to make a knockout pig. You start out with a fetus, you derive a cell line, using in vitro culture you target one of the clones, you do nuclear transfer to create the heterozygote pig, and you do another transfer to create ear fibroblasts. Create another fetus cell line, create another clone, and ultimately you end up with 15502, which is a Gal-knockout pig. And that's actually the pig which was presented by both David Sachs and was on the National Resource slide as well, which was the first pig which we produced which was a Gal knockout.

Now, for those of you that are not familiar with molecular biology, essentially this is what equates to a "bar code" of PERV. Every band here is an individual copy. So you can see there we're looking at PERV-A in this blot. The left-hand side is the original fetal cell line; the right-hand side is the 15502, the knockout animal. And you can see here we have about 10 copies of PERV-A, 10 to 20 copies of PERV-A. The pattern looks the same in the left-hand lane as it does in the right, so there's been no alteration in the PERV-A profile.

However, if we look to PERV-C, the virus which can infect pig cells, we do see a difference. In going from this point here through all of these procedures to the knockout pig, during the production of this pig, it's acquired two new PERV-C loci completely novel to that pig.

Obviously that was very interesting, and I'll just try to simplify this slide as far as possible. And essentially we looked at the various stages in the production of this knockout pig, 15502. Going from the fetal cell line to ultimately the first pig, which was 15045, you can see here that this new locus has developed somewhere in this procedure, either in vitro culture or the nuclear transfer procedure. Once that locus has formed, it's inherited down into the subsequenT-cell lines, as you would expect.

The important point of this slide is actually where we delineate where this effect occurs. In going from this fetal cell line here through to 15502 and the sister of 15502, we see that there's an additional locus come up, and that copy of PERV is in exactly the same position in the pig cell in both 15502 and its sister. So if the integration site is the same

there, it clearly didn't happen in the nuclear transfer, because this is a cell clone. So it must have occurred in the in vitro culture. So looking at—the potential here is that during the in vitro culture period, you can get superinfection of cells with PERV, potentially creating completely novel, completely unique PERV loci.

Now, that may sound a problem. The very encouraging news is it's really probably not a problem at all. The biology of the locus, well, it's probably going to be replication competent because we've proven that it transmits in vitro. We don't obviously know the effects that it would have on the host, or the potential consequences for the safety of using that potential animal ultimately, but because it's a unique locus, this can easily be removed by breeding. You simply just breed the animal, screen the offspring for that unique locus, and select the offspring which do not have it.

That is all dependent on the new locus behaving as a stable gene, stable mendelian element, and that's exactly what we see. In the offspring of 15502, we see that the PERV is inherited, just as you would expect any other gene, and thus you can clean it out of any donor animals quite easily.

Two sort of summary slides touching very briefly on publications which I think are prudent to know about when producing a report, this was published in *Virology* in 2003. A concern that was raised quite frequently was that PERV may recombine with human endogenous retroviruses. Everyone here in the room, about between one and eight percent of our genomic DNA is viral.

This is a study looking at essentially the major groups of human endogenous viruses that have potential for biology if they were to recombine with PERV. You can see here from the right-hand column, the potential for PERV and HERV even to be co-packaged, which would then have to—is the first critical step leading on to recombination, is incredibly rare. So in essence, the potential of PERV recombining with HERV can really be taken off the radar screen here for good.

Another paper which is—a couple of papers which are in press from out groups in *J Virol* at the moment is the potential for human cell infection is determined by exogenous forms of PERV. So far the field has been thinking along the lines of germ line elements which are inherited by pigs, and therefore that's the primary source of human-tropic virus. That's no longer the case. We examined a transmitting miniature swine, so a miniature swine which transmitted A/C recombinants to PERV human cells in vitro culture. We did a thorough screening of the germline DNA of those animals, and we could detect absolutely no copies of replication-competent PERV in the genomic DNA.

As I said, if you look at the human cell cultures, you see these PERV A/C recombinants, so the assumption has been that when you look at infected—these two right-hand lanes are infected—cultures at the DNA and RNA level, that these recombinants, the PERV-A/C, as they're called, are coming up due to in vitro culture and recombination.

What our paper shows, which is out in print probably about a week ago now, is that if you look at the PBMC of the DNA of the animal—this is the pig's PBMC—for a single-copy gene, you don't detect the A/C recombinants at all. So the A/C's are not endogenous. That supports the mapping studies. However, when you look at the RNA of the pigs, you can clearly detect these A/C recombinants in the pigs at the RNA level; i.e., it's an exogenous virus.

Clearly the future studies which I think are important, what's the mechanism driving the formation of the A/C recombinants? Is it a purely exogenous virus that is just hopping from pig to pig to pig? If it is, in that case it will most likely be probable to remove it from the donor animals by the various derivation techniques which will be used to get rid of the other potential pathogens which were mentioned earlier today.

The other scenarios that may be generated by recombination in vivo. Obviously PERV-C plays a critical role there; PERV-A is pretty ubiquitous throughout—or is ubiquitous throughout the pig species and lines. PERV-C is not. So with PERV-C being a critical component, you can obviously work with animals which do not possess the PERV-C.

The possible clinical significance, I think this is the critical issue which needs to be looked at now with respect to ultimately clinical safety, is that depending on the mechanism, it's quite possible that non-transmitting tissues, when they were transplanted as non-transmitting, they could, if these recombinants were generated sometime after transplant in the xenogeneic setting, become transmitting-type tissues. Whether the transmission would occur, I

think the possible post-receptor blocks that Dan Salomon and Carolyn Wilson are looking at are absolutely critical. Again, I think it's most likely that we're looking at one of these scenarios probably up here, from all of the data, and I think it will ultimately, again, be a very addressable issue.

And lastly, but certainly not least, I would just like to thank the collaborators, both the Sachs and Sykes laboratories at the Transplantation Biology Research Center at Mass General, the Prather Laboratory at Missouri, who produced 15502, the pig that we've been seeing in many presentations, and also at Mass General, the laboratory of J. Fishman.

And once again, thank you for the opportunity to bring the science up to date.

DR. VANDERPOOL: We have a few minutes for discussion of all three of these papers and their possible bearing on the State of the Science report, so feel free to come forward with your questions to Dr. Salomon, Sykes, and Patience.

DR. SWINDLE: Yes, Dan I want to pursue a little more information on hepatitis C. Hepatitis C in pigs is an essentially non-pathogenic and innocuous type of infection, but I'm not clear on pathogenicity in humans from hepatitis C and whether there are any food chain agricultural types of associations with the transmission.

DR. SALOMON: I think the honest answer would be that's probably beyond my expertise. We invited Dr. Meng to contribute to the book on it, and of course I read it, so I can come back to you with what I've read. I just want to make sure that's clear.

So from my understanding, the two things is that it's known to be xenotropic; it's been passed to non-human primates, for example, and causes infection in non-human primates. And I think the interesting thing that I tried to bring out in the slide, Michael, was at least the beginnings of molecular evidence for association of human strains of HEV in areas that had molecular identity or similarity—I shouldn't use the word identity—to local swine strains, which is the beginning of a molecular argument that there was transmission.

And I think that's about—Carolyn, do you want to comment on that further?

DR. WILSON: As I recall, it is pathogenic in humans. It causes, I think, a type of severe diarrhea, and in regions of India where it is endemic, there are certain [*inaudible*].

DR. MICHAELS: I think it's more akin to like a hepatitis A. It's very rare to cause a chronic hepatitis, but it can certainly occur in humans. There has been increased antibody conversion in individuals that do work with pigs where it's been studied. But it's not a chronic hepatitis, it's more a GI organ.

DR. SALOMON: I think to put it into perspective, Michael, my main take on it is that according to the studies, it's present in 80 to 100 percent of U.S. swines, pigs. And so it's there; we need to screen for it, we need to be very aware of it, and any sort of designated pathogen-free colony just needs to be kept free of it. And the fact is that it will be a job to do, because there could be transmission in either direction.

DR. ALLAN: Dan, do you know if it's a pig origin or human origin; in other words, chicken or the egg? Did the humans give it to the pigs?

DR. MICHAELS: This is not an answer to John's question, but germane to the conversation. Before hepatitis C was recognized and there were some SPF porcine colonies, when they went and studied those colonies, just by chance there were some of the colonies that had been put into a pathogen-free environment that happened to be negative at that point. And so they maintained negativity, whereas the ones that because they weren't screened for that particular microbe, that if it was already in there when they were put into their isolation quarantine procedures, they continued to maintain it. But it was intriguing to see that once that organism was identified.

DR. SALOMON: Something John said was interesting, because I think we always ought to seek to put all this stuff into context. So one of the things that to me is so amazing is this overwhelming evidence that infections occur in animal populations and move into human populations. But that's a very "human-centric" view of things.

So what John is saying, and the discussion that kind of comes about, is that it's perfectly reasonable for us to take the opposite view as well; that there are infections in human populations that are moving into animal populations to their detriment as well. So the idea that it's a two-way street is really well taken.

DR. ALLAN: A little bit of a follow-up to the—you showed one slide, I think it was the New Zealand group that has, I'm assuming, state-of-the-art diagnostics for a whole series of porcine infections, and what I'm wondering is, is that being translated into colonies like in this country for SPF or whatever you want to call it, and even for this new resource? Are we going to be using state-of-the-art diagnostics for these new colonies?

DR. SALOMON: I sure as heck hope so. I mean, that would be the function of doing good science and getting it published in peer-reviewed international journals.

Dr. Chang, are you here still? I knew you were over there, and I don't see you over there now. It would be interesting to ask. I think that overall, if you look at that report and the very nice review by Pin Paul and his group, is that the pig pathologists and virologist are very aware of this. But you're absolutely right, that would be the test.

DR. ALLAN: Like of the mini-pigs that are being used now and their knockouts, are they free from all of these non-pathogens that we know of, or do they have some of these things?

DR. PATIENCE: Certainly they're free of—I'm not going to make the list, but all of the organisms that we're worrying about here, CMV, for instance, HERV was not in those—

DR. SALOMON: Hepatitis E virus?

DR. PATIENCE: We've never detected hep E in any of the herd at all.

DR. ALLAN: And herpes and circo?

DR. PATIENCE: Circo I would have to check, to be honest, but I'm 80 percent sure that they were circo negative as well. But I'll check that.

But again, the Gal-knockout animals have not been produced in an SBF type environment, and again we're just looking at an elevation to an SBF-type pig to achieve the removal of the organisms.

And coming back to the question of is it state-of-the-art technology, well, yes, it is. I can assure people around the table that really it's being led by—the academic, the biotech, the food stuff industry has incredible tests for most of those organisms now.

DR. VANDERPOOL: I think one of my functions is to ask lay questions that may interpose a degree of ignorance, but I'll ask them anyway. To try to ask something in ordinary terms, with a bit of historical background, just a few years ago we were worried that closed colonies or whatever we wanted to do—whatever measures we could take could not breed out PERV because it was seen as endogenous virus within the DNA. Now, isn't it true that Dr. Patience, your study and some of what the others of you are saying is that for this species of swine, PERV is not endogenous, it's exogenous, and therefore can be bred out of a colony and therefore you don't have to worry about PERV? Or is that just way too simple-minded?

DR. PATIENCE: It's potentially not too simple-minded. What we've described is a thorough screening of an animal which transmits PERV to human cells. We screened the genomic DNA, and we found no full-length replication-competent virus there; it was all mutated in one way or another that rendered it replication incompetent. The virus that we did find was the exogenous recombinant variant. Now that is going to be the issue to deal with next.

If you review the literature, actually quite interestingly, on the porcine endogenous retrovirus, and look at the papers which have described PERV that's isolated from the genomic DNA of pigs, there's very, very few copies that are really replication competent. Ninety-nine percent of the copies are replication defective. So it actually looks to be a much easier issue to develop pigs which don't contain loci which contain replication competent copies than we

thought back in 1997 when we first published "Human Cell Infection," and '98 when Carolyn Wilson followed it up with "Primary Cell Assays."

So I think the generation of pigs in the germ line which do not contain replication-competent PERV is very achievable, and now we need to sort out the exogenous angle.

DR. VANDERPOOL: And the message that would follow from that is the degree to which PERV could not be bred out, PERV does not seem to be the threat we once thought it was. Is that fair, too? Dan, is that fair?

DR. SALOMON: I think what I said kind of stands on its own. I think that until you have good scientific data that convinces you there's a real disease being produced here, it's appropriate to be cautious and not raise unnecessary alarms.

So right now, I think that's probably close to being correct. I think the key thing here is to realize, again, that you have to keep this all in context. PERV is a possible risk. By studying it over the last seven, eight years now, the knowledge that we've gained in those studies have taken this and put it into perspective, and we've created strategies now that have been reviewed to move it out. I mean, to me, that's a triumph of what science is supposed to do.

So I think that this is the process that we have to go through. Now we need to go and say, are there other unknown viruses in the pig genome. There was one paper that I didn't think was good enough, really, to highlight, but one of its conclusions was that there were a whole bunch of unknown—currently unknown, I should say, retroviruses that were re combining with PERV in the pig. So the story is not really over yet.

DR. VANDERPOOL: Right. The last thing you want to do is overstate, but at the same time, the last thing we want to do is maintain some scenario that is more alarmist than is warranted.

DR. SALOMON: I should just say, I've been very clear now for almost five years that in my personal opinion, based on everything I know—and it hasn't changed, it's only improved—is that we can move forward to carefully planned, extremely well-monitored xenotransplantation trials in the clinic.

DR. VANDERPOOL: Dr. Sykes and then Dr. Patience.

DR. SYKES: Clive, if I've understood you over the years, the non-transmitting herd of pigs in fact does not make the A/C recombinant that infects human cells, so we already have a line of pigs that we don't need to worry about this with. And I think the data that we've shown today, where you have for many months human and pig cells coexisting at high levels in vivo, and indeed that non-transmitting pig has not been able to directly infect those human cells, is highly consistent with that. Is that correct?

DR. PATIENCE: I think that the studies are consistent. If we look back from when David first started to derive the inbred miniature swine, we've now screened the genomic DNA and we don't see any replication-competent copies. We do actually think that our molecular techniques to detect the A/C are more sensitive than co-culture techniques, so this has only, again, improved our level of security to look for these human-tropic viruses.

But I think, again, it's a thorough screening of the genomic DNA of the animals which are going to be used to derive your clinical herd, followed up by monitoring for the A/C recombinants, for instance, and the other porcine pathogens is what's required. But I think you're right, it's a consistent story, a consistent evolution of knowledge which, to reiterate Dan's points, is only serving to increase the safety that we're going forward with.

And if I could, without turning the microphone off, just to mention the study which Dan made reference to was a sequencing study of a number of genomic PERV copies, which was really a repeat of studies which we performed about three years ago. They're all highly defective. They show clearly evidence of re combination within families, but the other families which are reported have no potential to infect human cells. There are multiple, multiple point mutations that really rendered them inactive.

DR. ALLAN: So what you're also saying is, is that in these mini pigs you wouldn't expect to see these A/C recombinants? In this line. You wouldn't expect to see these A/C recombinants in this line of mini pigs?

DR. PATIENCE: In certain non-transmitters, as I said to Megan, we get better sensitivity out of the molecular assay, so I think by the in vitro co-cultures you probably miss some transmitting animals.

Now, knowing that the A/C is defining the human-tropic nature, if you can clearly take the non-transmitting animal, screen them for A/C, if they turn up negative, then I think you're in an incredibly strong situation.

DR. ALLAN: Since the A/C's are exogenous, you can't really screen the genomes for these pigs, you sort of have to look for the virus?

DR. PATIENCE: You have to look for the virus. You have to look for the RNA, you have to look for the source of the trouble, which again, the elevation and sensitivities with the molecular techniques just only serves to benefit you in that way. What I think is critical is to understand the mechanism of generation of those A/C's. So it may be that in the germ line DNA for the 'ultimate clinical product,' in inverted commas, you may want to remove the long but incompetent copies of PERV as well from the genomic DNA. Which is, again, feasible by breeding, feasible by knockout technologies, but you have to identify which copies you may ultimately want to knock out as you develop a safe and safer product.

DR. ALLAN: Not to get too technical, but it seems like if all of your PERV's are defective, the likelihood that you're going to get a replication-competent recombinant is going to be very small because you don't have a virus to rescue and to create a recombination event.

DR. PATIENCE: I think that's true. For those not fully versed in the virology, I don't blame you, but the probability of generating a recombinant is increased if there's a replication-competent virus which drives the event. If you use an animal which does not contain replication-competent C, for instance, and only has defective germ line elements, I don't think it gets much better than that.

DR. SALOMON: I just want to make one last comment just to make sure that this very high-level discussion of PERV-free animals doesn't, as usual, turn up in the lay press as "there's no such thing as PERV and it was all made up as an evil conspiracy" or something. I mean, the truth is that we're doing pig islet xenotransplant experiments with outbred swine, and every single one of them have infectious A and B PERV virus.

So I just want to make sure for the audience that didn't follow all these details, that if you go to your local pig herd, a large number of those animals are shedding infectious virus that go into the human cells. So these are very highly screened, special animals, and it's very exciting, of course, because they chart the future for commercial application and clinical trials, but I just want to make sure that everybody gets the idea that you can't just go out to your local farm yard and get a healthy pig.

DR. VANDERPOOL: Dr. Kaslow?

DR. KASLOW: I think they've answered my question with this last discussion. It basically sounds like you can't conceive of a situation in which the A/C recombinant could rescue one of the other—or an unknown defective virus, at least of the defects that you've seen so far. Is that a fair interpretation?

DR. PATIENCE: I think it's thoroughly encouraging that we're going to that level of risk, yeah, that we're thinking of a recombinant being generated which rescues another defective, et cetera, et cetera. And I think some of the other—get moving away from the germline, some of the actual analysis of primary human tissue is going to be critical to determine whether primary human tissue can even be infected.

DR. VANDERPOOL: Thanks so much. We need to move on to the clinical trial in Mexico City, and we have two presentations with respect to that, first by Dr. Rafael Valdes, and then his coworker, Dr. David White, of the university of Western Ontario.

DR. GROESCH: So our first speaker is Dr. Rafael Valdes. Thank you, Dr. Valdes.

Agenda Item: Overview of Clinical Study in Mexico City: Islet of Langerhans Porcine Xenotransplants in Type 1 Diabetic Patients

DR. VALDES: Well, thank you very much, Mr. Chairman, and to the Committee for the opportunity to talk here. It is my privilege. We'll talk about our results in two different clinical trials. Well, this is a pig-to-human islet xenotransplantation as a treatment in adolescents with Type I diabetes. We'll see some background, ethics, transplantation technique, results, and safety.

These are some of the works done years ago using kidney as the xenografts, using baboon, pig, goat, sheep; heart transplants, liver transplants, and there is a very important work done by Hal Groth in the '90s. He transplanted 10 patients with end-stage renal failure, and he transplanted islets coming from adult pigs; eight into the portal vein, two in the renal capsule. And those patients receive chemotherapy, prednisone, cyclosporine A, and acetopyrine, and they were not able to measure for a long period of time porcine C peptide in urine. They didn't have any clinical improvement from the diabetes point of view, but at this time those patients haven't had any evidence of PERV infection.

Let's talk about Sertoli cells. As you well know, the test was described as a privileged place in which we could transplant T-cells, so Dr. Selawry started looking at what happened with the transplants, and they found that the Sertoli cells could play an important role in these protection.

Let's talk about the potential benefits to the patient must outweigh the potential risks. In Mexico, as you can see, we have 12.8 percent of our population has diabetes, and 10 percent of them are diabetic type I. Comparable to those here in the States and in Europe, in Mexico, diabetes is the first cause of death.

And let's just have a look of the risks of diabetes. Despite the tight control and monitoring diabetic patients, most of them will develop blindness. It's the first cause of blindness in my country, it's the first cause now of renal failure in adults in my country, and all the other things who produce the diabetes.

What's the risk in xenotransplantation? Cross-species infection. What's the advantage of our procedure? Unlimited availability of high-quality donors, it's a very simple surgical procedure. That means we do not touch any vital organ and we don't use any chemical immunosuppression. So on a balance of this ability to cure diabetes by xenotransplantation, the absence of chemical immunosuppression seems to outweigh the risks.

Our protocol was submit, and it took almost one year to get approval the project, of the protocol and the informed consent. They were sent to our research boards at the hospital and at the National University of Mexico, and we had some modifications at that time. Then we got extent (*sic*) consultants. We have the approval of the National Transplant Center, and after that, we had a review of our National Bioethics Committee.

How was the patient selection? We took patients of either sex attending our diabetic children's clinic at the Hospital Infantil de Mexico Frederico Gomez with a mean age of 14.7 years, with type I diabetes diagnosis two years, at least two years, before to get involved into the project, glycosylated hemoglobin between 8 to 13, insulin requirements 0.8 to 1.5 units per kilogram per day. We made the human C peptide less than 0.8 without signs of chronic complications from the disease in order to follow the patients and to ensure the proper microvascularization of the device.

We had a psychological evaluation for compliance. All the patients had to be Mexican residents, attend several informative meetings, and sign informed consent letter following the Helsinki declaration.

This is the device that we implant into the abdomen under the skin. We use to do a very small incision through the skin and then to implant the device just under the skin, so the procedure is very easy. The device is made of stainless steel and Teflon.

Then we implant the device and we leave the device for two months in order to get collagen as well as new blood vessels. We take neonatal pigs seven days old, and we remove the pancreas, we process the pancreas, then we

remove the testes and process the testes, and we put all together into the device after two months of the original implantation.

Here is how the devices looks before the cell implantation. That's the isolation procedure for pancreas. Here is the way that we used to do the ditizone staining for purity, acridine orange and iodine propidium for viability, and we do simulation and glucose index which has to be more than three.

Then the testes are—we use liberase instead of collagenase. Here you can see how the Sertoli cells look. We use to do Vimentin stain for purity and acridine orange and propidium iodine for staining viability. So after cell isolation, a sample of mixture is sent to microbiology laboratory of the hospital and tested for fungal, bacterial, and parasitic sterility by different medias.

So we did two clinical trials; the first, the cells were flown in from New Zealand to Mexico and mixed in Mexico the islets and the Sertoli cells before transplantation, having just three days of culture. The second trial was 10 patients; all biological materials were processed and obtained in Mexico, and the cells were co-cultured for 14 days.

Here is the result of the first group, in which you can see how six of the patients reduce the amount of insulin requirements after transplantation. Here is the insulin requirements, here is the first transplant, second transplant, and two of our patients received a third transplant, looking to leave them free of insulin.

And here you can see before transplantation the insulin requirements; then posttransplant, 97 percent, 100 percent, and the very important thing is the glycosylated hemoglobin dropped in all the patients. That means that the metabolic control is very much better now.

The second group we had a peer review rejected the data on the grounds that insulin reduction could be due to close endocrinological monitoring of the patients. In the second group we institute a long-term 10 months pretransplant baseline with close monitoring. In here you can see the baseline, and something that we found is that not all the patients has to be reduced the insulin requirements with a very strict metabolic control. Some of them, as you will see, increase the amount of insulin requirements.

So this is the group who reduced insulin requirements, and here is a graph explanation. You can see starting insulin requirements at the time of entering to the protocol, then how the insulin increase with a strict metabolic control, and then how it reduce after transplantation. And the same happened with glycosylated hemoglobin.

These are all the patients who reduced, had no reductions, and six had reductions. Those patients are now six months after the first transplant, and we have re transplanted some of them in order to live free of the insulin.

Here you can see in all of them how the glycosylated hemoglobin improves very much. Here is something that many of the journals ask for us to show, which was the cells in the device. In here you can see after 2.5 years, the cells and the collagen. This is the inside of the device and this is the positive stain of the cells for insulin.

So we have been talking about pigs and the safety of the animals; safety measurements during all harvesting and processing, the patient follow-up of PERV, and what is the contingency plans. Well, that's the way that our farm is taking care of the pigs. The farm is located in the north of Mexico in the state of Sonora, and this farm has the compliment of the international regulations for growing pigs free of any evidence of infection. The animals are coming to our lab, and they have to have first a hot bath in order to keep warm the animals, then we use a surgical soap, we cover the extremities, we took serum samples, we take small pieces of heart, liver, spleen, and kidney of each individual pig, and archive both frozen and fixed. And humane treatment and euthanasia of all animals.

That's the safety measurements of the animals at the hospital. So microbiological screening of blood from sows and donors are carried out by the faculty of Veternaria at the University of Mexico, and the Forest Agriculture and Farm Research Institute Mexican government. Those are some of the screening in the pigs. You can see here circovirus.

And let's see what our facility looks. We have a very secure lab, one with flow-through system, air gradient 99 percent. Here is the isolation area, here is the operating room just for pigs, and those are our lab for PCR. All the concerns with PERV infection in vitro of some types of human cells that we have seen, the discovery of PERV-A

receptors in human cells, but what's in vivo, not a single human case reported worldwide, despite millennia of pig human close contact and extensive clinical use of pig-derived medical products now show no content of PERV. All long-term non-human primate studies so far have failed to induce in vivo infection even with severe immunosuppression or cell-free particles plus infect T-cell infusions in large quantities.

This is our follow-up in the patients, the PCR and RTPCR, which we can see the positive in the pig; the patients are negative. So no complications or infection have been found for a follow-up of longer than three and a half years.

Contingencies. Detail of contingency plan is in place in case you have withdrawal. We have to test the patients for serological or clinical evidence of infection, test blood and tissue samples from donors for possible infection.

So these xenotransplantation techniques holds some promise for the treatment of diabetes type I. Clinical trials must continue in order to determine best inclusion criteria, to predict better results, together with experimental models for improving the technique.

And I would like to thank our friends who collaborate with us, Dr. David White, Dr. Camilo Ricardi, and Dr. Luke Anvernadi, Dr. Gordon Weir, and Dr. Robert Elliott, the Mexican Health Services, National Science and Technology Council, the National Autonomous University of Mexico, Patrinato (*inaudible*), and Interneda (*inaudible*). Thank you very much.

As Dr. White is going to present another part of our work, so I will beg you, if it's possible, to have the questions at the end Dr. White's presentation.

DR. GROESCH: Sure. Thank you very much, Dr. Valdes. And now we will hear from Dr. White on the immunological findings with this study. And Dr. White is from the University of Western Ontario.

Agenda Item: Immunological Findings in the Mexico City Trial

DR. WHITE: Thank you very much. My laboratory has been collaborating with Rafael Valdes now for a bit over three years looking at the immunological responses that are generated in his patients, and we routinely in my lab measure antipig antibodies, essentially by analyzing pig rad cells. We also look specifically at anti-Gal IgG and IgM, and more recently we've taken also to measuring antibodies against porcine C peptide. Because of time restrictions, I'm just simply going to concentrate on the anti-Gal responses, which I think give the main message. The anti-pig antibodies are useful, in that by doing subtraction or absorption studies, you can actually measure non-Gal antibodies. More recently with the Gal knockouts, we have better techniques now for doing those kinds of studies.

So just to remind you, in the first trial there were 12 patients; 11 of those patients were re-transplanted. The islets and the Sertoli cells were actually shipped from that clean farm of Diatrans in New Zealand. I'm going to express the results for you as the mean titer of all the patients. If I showed you each individual patient, it just looks like a pretty colored rainbow.

And just to be slightly immunologically controversial for a minute, I'm going to work on the assumption that the IgM responses we see are a surrogate for B-cell responses, and the IgG responses we see are surrogates for T-cell responses.

So let's look first of all at those anti-Gal IgM's. Just as an aside, the pretransplant titer of the anti-Gal in these patients is somewhat higher than we see in our standard control serum populations. Our standard control populations actually came from the transplant group who work at the University of Western Ontario, and their titer would be on average about 1 in 30. Here you can see we're 1 in 90. We asked ourselves the question, is this because these patients live in Mexico or is it because they're diabetic, and it turns out that it's because they're diabetic. If you actually take diabetics from London Ontario, you get much the same titer.

You can see that seven days posttransplant, that titer has actually gone down, presumably because there are Gal-containing proteins or what have you leaking out from this graft which, after all, has now traveled halfway around the world from New Zealand, but by day 14 and by one month, it's quite clear that all of these patients have

mounted an immune response to the Gal. By six months, when they're coming up for a second transplant, that Gal has subsided once again to background 1 in 90, and you get exactly the same response at one month post the second transplant as you do for the first. So clearly we haven't expanded the B-cell population in any way.

Now, I've been talking about these patients making an immune response, but actually if you go from 1 in 90 to the maximal, which is about 1 in 180, that's only a doubling of the titer. That's one log. And we really kind of wanted to know what would have happened if you had done the transplant without the Sertoli cells. Well, we didn't, but as Professor Valdes has referred to, Hal Groth back in the 1990s did do transplants with pig islets into patients; the techniques were different, the islet source was somewhat different, and in the Hal Groth group, he gave them fairly aggressive T-cell, at any rate, immunosuppression. And despite those differences, I just thought you would be interested to see a comparison in the immune response in the two patient groups.

Here are the group from Mexico, with that doubling of titer, and this is the response published by Golili using the same assays as we do to anti-Gal IgM; a significant, statistically significant, for those who like statistics, difference between the two groups. Interpret that how you may.

Let's move on to the IgG's. And the IgG's we saw an entirely different message from the IgM's. We saw a very substantial response to that first transplant, going up from about 1 in 120 to something over 3,000. But interestingly, that response fell quite rapidly posttransplant, and on second transplantation, although there is a response, it's actually diminished compared to that first response. And at one year they're back to background. Just for completeness, here's the comparison. Once again, it's now IgG's between the Mexican patients and those transplanted by Hal Groth. The difference here is actually not statistically significant; the deviations were quite large.

If you look at responses now, as opposed to absolute titers, so what you're looking at is increase on a log scale, you can see that the IgM responses in these patients really are fairly pathetic, one log, essentially; whereas with the IgG in the first group—the first transplant, rather, you see a response of about four logs; whereas with the second transplant, it was 1.7 logs. Again, if you like statistics, the difference between the two is statistically significant.

I think this may have immunological significance. And in order to explain that, I need to give you, as it were, 101 Sertoli cell immunology. Now, Sertoli cells actually secrete all sorts of interesting and immunomodulatory proteins. They secrete clustering, which inhibits complement. I'm very keen on inhibiting complement. It's secretes TGF-beta, which is immunomodulatory. They secrete insulin growth factor, which could well be stimulating the regrowth of stem cells from a less-than-effective transplant. So there's a lot of proteins in there that we have to actually work out what their overall role is within the Sertoli cell effect that's been described in literature by so many.

But I want to concentrate just on one aspect. This is my attempt to draw a Sertoli cell. And Sertoli cells secrete, have on their surface, this *fas* ligand. Now, you transplant that Sertoli cell, and along comes a T-cell, and the objective of this T-cell, T-lymphocyte, is to reject pig. And when it sees pig islets, pig Sertoli cells, this T-cells gets very angry, it gets activated, and it expresses *fas* on its surface.

So what now happens when *fas* and *fas* ligand interact? Well, what happens is you get something called apoptosis; the T-cell goes, as it were, all into a spin, it receives a signal by the interaction of *fas* ligand, and it goes away and it commits suicide. That's what apoptosis is, essentially.

Now, if that were happening in these transplants, we would want to look for some evidence that there is a T-cell loss in these patients. Unfortunately, at a distance, you can't do mixed lymphocyte cultures. Now, when we actually measure the antibodies, we measure them blind; we don't know what's happening to the patients. When we actually broke out the code and we looked, and we split the data that I've just shown you into two groups, one group who actually had some evidence of function as demonstrated by reduction in insulin requirement, and the other group that did not. And you can see at one month, the successful group—if I can call them that—have a major increase in titer; in other words, a bigger T-cell activity compared with the unsuccessful group. But when you go to the second transplant, you can see that the IgG response is now much reduced compared to the unsuccessful group. That is significantly different.

So there is just some indication that we are indeed seeing a T-cell deficit in those trials. Clearly to demonstrate it, what you would want to do is to compare it with patients who just have islets alone, which you obviously couldn't do.

Let me move very briefly to the second trial, which is still very much in progress, 10 patients, as you've heard. Essentially the technique is the same for Trial One, except that the islets and Sertoli cells were now co-cultured for 14 days prior to transplantation. And the pigs actually came from the pig improvement company based—or their facilities based in Mexico.

So now if we look at the immune responses in these patients—oh, just to say a word about co-culture. It's been demonstrated over and again that if you culture islets for a long period of time, they lose a cell population called dendritic cells and they down-regulate the expression of MHC. What that means in simplistic terms is that they lose antigenicity, they lose an ability to stimulate a direct presentation, to give it the buzz words.

So now if we look at the IgM responses, if anything, they're even worse or even lower, even better than in the first trial. The titer in these patients was slightly higher, about 1 in 120, again going up to 1 in 180, not even a one-log shift. And by the time of the second transplant, you're really getting no response there at all, although these were measured very recently. As I say, we're only about six months out now, and I'm not quite sure what's going on the slight increase in titer out here, and we'll have to watch that. But a very low IgM response; if anything, even lower than the first trial. Not surprising, given that the islets have been co-cultured and they're antigenistically reduced.

If you look at the IgG titers after the first transplant, the message really is the same for IgM. The immune response—let's take one month—is really not that great. You're looking at a titer of 1 in 500. If you remember in the first trial, at one month our titers were somewhere up here. So completely different picture. However, at the second transplant, you now see a much bigger response than the first transplants, so completely the converse of what happened in the first trial. And again, if you look at this as a log scale, you can see the IgM responses in these patients are really not great; the IgG responses are bigger after the second transplant than the first.

And if I can just put the two trials side by side, IgM's, essentially the same. It's a one-log response in the first trial and it's a half a log response in the second trial; essentially very weak IgM responses. But with the IgG's we see quite a difference; a four-log response after the first transplant, whereas only a two-log response in the second trial, and a 1.7 log after the second transplant, whereas much bigger responses, three and a half logs.

So if we compare the two trials, in both studies we see a very weak IgM response. The IgG responses using the original technique give a higher response after the first transplant, and then in trial two we see a higher response after the second transplant. The turnaround. I believe that what we're seeing here—in the second trial what we're looking at is primarily an indirect presentation. Most of the interactions are going on within a distant lymph node; the recipient antigen-presenting cells doing the presenting, as opposed to the donor antigen-presenting. And, of course, the question I would like to know the answer to but I can't tell you, is do these differences actually make any difference two overall graft survival? You've seen the survival results at this stage, six months after the second trial; there doesn't seem to be a significant difference in the overall survival. If anything, the second trial, it's got six out of 10; the first one had six out of six. It's not statistically significant, but we can't see an obvious and dramatic difference with the two techniques.

So to conclude, I think that xenotransplantation of porcine islets and Sertoli cells would seem to hold some promise for the treatment of diabetes. The technique is extremely low risk, and as you've heard, there have been no complications to date, and immune responses elicited do seem to be technique dependent, and we're looking forward very much to the tests that are currently going on in Rafael's lab and starting up in my lab to actually confirm some of these hypotheses that we've derived from the clinical data when we do the animal studies. Thank you.

DR. VANDERPOOL: Okay. Comments and questions from the Committee, including ex officio members, for Drs. Rafael and White? Dan?

DR. SALOMON: That's a very nice set of presentations. Thank you. So the question I had, Dr. Valdes, is what kind of data, if any, do you have for porcine C peptide measurements? And that's an interesting area because of the

possibility of developing an immune response against the C peptide so that you don't find the C peptide. So these are the kinds of questions I want to hear you respond to.

The other question, then, is if you can't measure porcine C peptide—I'm not saying you can't, but I'd be interested to know, can you. But if you can't, then the next question would be, did you do any stimulated insulin release assays as a way of documenting the islet function after the transplant so that you might have something pre- and post-transplant to compare it to?

DR. VALDES: Well, thank you very much, Dan, for your question. I think it is very important, and it was a real problem for us to show how the graft works. So first we took some samples of the patients, and in the first clinical trial we were able to demonstrate porcine C peptide temporarily. That means for two months, no more, 12 weeks.

After that we were really worried, because the insulin requirements was almost nothing in the patient, and we were not able to detect any porcine C peptide at all. So at this moment we are working on insulin and porcine insulin using different techniques, and I cannot tell you because it's confidential now, this. But anyway, the results are very encouraging now in our patients.

And on the other hand, David made some measurements of antibodies and porcine C peptide, and he found in some of our patients levels of these antibodies.

DR. WHITE: Can I just add to that? The disappearance of the porcine C peptide is, you would think, evidence of the death of a graft. And based on some work that Ollie Cosgran has done, there is also evidence that at least mice make immune responses which clears porcine C peptide. We developed an assay to measure specifically porcine C peptide, and we screened all the patients. We find on occasions antibodies in those patients to porcine C peptide; really quite a low titer. In my view, those antibodies do not explain the disappearance of porcine C peptide continuously in all the patients. It doesn't seem possible that it's as simple as immune complex, even though we do find some antibodies.

And as Rafael mentioned, the thing we would really like to do, of course, is to be able to demonstrate the presence of porcine insulin after transplant. Now, porcine insulin differs from human insulin by one amino acid. It's a lutivale, which is 32 daltons different, which is a bit vicious to actually be able to detect the difference between human porcine insulin, because most of these patients are injecting insulin, though not all of them. We do have now mass spec techniques which allow us to actually separate on the basis of molecular weight human and porcine insulin, and hopefully by the next time this committee meets, we'll have screened all those patients and be able to demonstrate the presence of the porcine insulin. Because that's clearly what this study means.

DR. SALOMON: I mean, the other thing one could do, given that, at this point, current available ELISA cross-react with pig and human insulin, is that you could do a stimulated insulin release assay before the transplant and then follow it up after the transplant. And then I think most of us would agree that the difference would be attributed to the function of the pig islet graft.

DR. VANDERPOOL: Dr. Sachs?

DR. SACHS: Have you done any studies in streptozotocin-treated baboons or any other non-human primate where you could ask some of these questions and be able to look at the actual islets?

DR. VALDES: Well, actually, we are working with Dr. Ricardi with monkeys, with baboons—not baboons. I mean, yes. And I don't know, I think they inject a streptozotocin to the animal.

DR. SACHS: But you don't have any results you can share with us from the monkey studies?

DR. VALDES: Okay. No. No. No.

DR. SACHS: Can I ask one other question?

DR. VANDERPOOL: Sure.

DR. SACHS: In the data on the patients in the first trial, they were divided into two groups. Was that post hoc; after you saw the data, then you just divided them on the basis of which ones looked like they responded and which ones didn't, as opposed to any other criterion for making that separation.

DR. VALDES: No, no, no. We just took those who respond and those who didn't respond. And the question at that time was giving a very strict metabolic control to the patients, some of them can reduce 30, 50 percent less of insulin requirements. So the claim was perhaps those patients are not working, and the decrease of insulin requirements are because they have very strict metabolic controls. So that's why we changed our plans and we put the patients on 10 months on a very strict metabolic control, then the transplant.

DR. VANDERPOOL: Dr. Valdes, just a question that comes to mind. Have you had any adverse events from what we call in the States adverse events, namely patients—

DR. VALDES: Complications?

DR. VANDERPOOL: —who experience unique sets of problems—

DR. VALDES: Not at all.

DR. VANDERPOOL: —or fever or something like that?

DR. VALDES: I'm talking about one infection. We haven't had any one infection. And we have been measuring CD-4 and CD-18 patients, and the patient doesn't have any difference in those populations of lymphocytes.

DR. VANDERPOOL: Dr. Sykes?

DR. SYKES: You showed us insulin staining on one implant removed, I think at two and a half years, you said. Why was that implant removed, first of all? Secondly, did you detect any Sertoli cells in the implant? And third, how many have you had the opportunity to—how many implants have you had the opportunity to examine histologically?

DR. VALDES: Well, at this moment, this patient is a patient who decide to give us one of the device in order to be re-transplanted. Because each patient has four devices, so we asked her if she wanted to be re-transplanted and said, okay, and we asked her to allow us to remove one of the devices in order to see if we could find some cells. So we found the cells.

We didn't have the final stains for Sertolis. The thing is that Sertolis and fibroblasts are the main two positive, so in the collagen, you can be confused with these kind of stains. So we are looking for another markers for Sertoli in order to show whether if there are Sertolis in the device.

DR. WHITE: Perhaps I could just add something to that. Because the pathology is actually being done in our labs now in Canada, and the issues really are yes to identify Sertoli cells, and there are no good specific antibody marker. There's a SOX 9, which works very nicely in mice and doesn't work at all really in pigs, and there's the Vimentin, which lots of other cells carry. There's recently been a publication of a specific probe which the authors call Strat, which may well give us a nice specific marker.

I think the important thing is to demonstrate the happenstance that these cells are genuinely pig cells. And these chambers, I should say, have only just very recently been removed in the last few weeks, and the obvious stain is to stain for Gal. But of course, as you know, islets are Gal weak or Gal negative.

In my hands, at any rate, if you stain a paraffin section for Gal, essentially the only thing that comes out positive is the endothelium. So we have to look for some other way. And what we're doing—and you might want to suggest whether it's the right thing to do—is we're actually using our PERV probes, and we're going to do in situ PCR for PERV to demonstrate the porcine origin of the cells. If anyone can think of another way of doing it...

DR. VANDERPOOL: Dr. Sachs?

DR. SACHS: Weren't you surprised that the porcine islets, the one you showed, it was very, very little in the way of viable—was it functioning?

DR. WHITE: No. Non-functioning.

DR. SACHS: This is one of the patients that failed?

DR. WHITE: Yeah. They wanted a retransplant.

DR. VANDERPOOL: Dr. Cooper?

DR. COOPER: A couple of quick points. First of all, was the timing of the second transplant different in the second series as opposed to the first series? It seemed to be much earlier. Was it then electively to be re-transplanted?

DR. VALDES: No, no, no. That's because we saw the immunoresponse in the patient, so we saw that after two months, the immunoresponse almost brought down almost to the same levels of the first transplant. That's why we decide to do after two months of the first.

DR. COOPER: And then in both your groups, about half seemed to respond and half didn't. Any idea why half seemed to—

DR. VALDES: Well, there are a lot of questions. You know, first, we are just thinking in Gal, and I do believe that there—

DR. COOPER: No, I'm not thinking of the antibody response, I'm thinking of the response of the reduction in insulin requirements.

DR. VALDES:: Yes, in insulin requirements. You mean some of them were—

DR. COOPER: Well, about half of them seemed to require less insulin, and half required the same amount as before. Why?

DR. VALDES: Why?

DR. COOPER: Why? Any idea why half did well and half did—

DR. VALDES: Well, in the first group that they didn't function, my belief is that we are looking just for the Gal, and there are some other pig antigens that we are not taking care of them very close, and I'm sure it's going to happen later. And in those patients in which we didn't get 100 percent function of the graft, perhaps it's the amount of cells. I mean, when you are doing this kind of work in a lab, you can get very good cells in one load and not very good cells in the second. And sometimes it depends on the liberase, for example, the load of liberase. It's different.

DR. VANDERPOOL: Dr. Collins and then Dr. Bloom.

DR. COLLINS: Thanks, Harold. Dr. Valdes, thank you for your presentation. In this country we've been pretty hesitant to transplant children who develop diabetes, because about a third of them over a lifetime will require dialysis, less than half will develop blindness in the course of the disease process, so we've struggled with the risks versus the potential benefits. Not everyone, when they first develop diabetes, will benefit from a transplant, is our thought.

With your studies, why did you select children to transplant? Is it to prevent the future complications of the disease? That's one question. The other point is, is the pathogenesis of the disease in Mexico different than here in the United States, or are there higher incidents of the secondary complications?

DR. VALDES: Well, the first answer is yes, we took the children because we wanted to stop all the damage of the disease. And your second question is related with the diabetes in Mexico. Our combination of Spanish and Indians make us more sensitive to develop diabetes than. Second, this is a very good news for you, we are the first Coke consumer in the world in Mexico, so it is really amazing the amount of Coke that we use. I mean, unfortunately the poorest people is the people who used to drink a lot of Coke with junk food.

DR. VANDERPOOL: Dr. Bloom?

DR. BLOOM: Thank you for your presentations. I have two questions. The first one has to do with what you know about the permeability properties of the device. It would seem to me, based on some of the comments that Dr. White made, that they're probably very cell permeable. For one thing, if you expect that the Sertoli cells are having an acetolytic effect on T-cells through the ligan fas pathway, that suggests cell contact, which means the T-cells and the Sertoli cells must be coming into contact. The other thing is, if you're suspecting that the remaining cells inside the device may not be pig cells, that also suggests you suspect certain permeability properties that indicate thaT-cells may be able to come in and out.

And then the second question I'll ask you afterwards.

DR. WHITE: Just to deal with the second question first, I definitely suspect those—well, the second part of your first question, I definitely suspect that those cells are pig cells. If you look at the H&E morphology, that there are obviously some inflammatory cells there, but they look to me to be the morphology of what I would expect an islet cluster, a pretty sick islet cluster, to look like.

The other thing that doesn't come out terribly well, but certainly from looking at a lot of pathology of the animal experiments that Rafael has done—most of these studies are pig islets in rabbits—it does seem as though the islets like to settle down in places of vascularization within the chambers. These chambers fill up with collagen, and it's difficult to prove, but one gets the sense that they're sort of moving, as it were, towards the most vascular part of the chamber, even going through—this wire mesh has got holes; you know, it is not a barrier. It's not an immunological barrier. The collagen is inside and outside.

Now, I can't quite envisage how an islet Sertoli cell cluster would actually migrate, but certainly from looking at the experimental pathology from the rabbits, it would suggest that they like to be close to the vascular part of the chamber, which sort of makes sense. So that sort of deals, I think, with permeability as well.

DR. BLOOM: Thank you. It sounds like the chamber is mesh and not intended to be impermeable at all.

The second question that I had was I was wondering if either in preclinical studies that you've already performed, in future preclinical studies, perhaps in the monkeys with Dr. Ricardi, or perhaps in future clinical trials, whether you plan to test pancreatic isleT-cells without Sertoli cells.

DR. VALDES: Without Sertoli cells? Well, we've tried in rabbits, but a rabbit is a very bad model, really. So we are looking to do perhaps some ducks using pig islets to see what happened. That's a big question. Actually, Roy Cal told me once, Rafael, perhaps your device works without any Sertoli. And we don't know at this moment.

You know, something that I can tell you is that we did some rats with a chimelo isografts using the device, and I can show you the pictures, the histology; you can see very clear how the islets are spread into the collagen. And the islets were not reject, but there was isografts in rats, no xenografts. When they tried to do xenografts with islets in a rat, the islets were rejected. Islets will succumb in the rats.

DR. VANDERPOOL: Thank you very much. I think you—Doctor?

DR. SYKES: I just have one more question. So in a sense, what is the longest that a single transplant has functioned, number one? And secondly, with the repeated—how many repeated transplants will you do? Will you just go on indefinitely transplanting these patients?

DR. VALDES: Well, the first question, our oldest patient is four years now.

DR. SYKES: From one transplant?

DR. VALDES: From three transplants.

DR. SYKES: So what is the longest that you think you've seen function from a given transplant?

DR. VALDES: Well, let me tell you something. This patient, she was in the first clinical trial. They started with the first transplant in 2000, and the third transplant was carried out at the end of 2000. So this lady has almost three years.

And the other thing that we have found is that we can reimplant into the device the cells. That means that the patients can be re transplanted without removing the device.

DR. SYKES: And how many transplants will you ultimately do?

DR. VALDES: We have done in the second group three grafts in each patient, and we don't know at this moment.

DR. ALLAN: Two parts of my question as well, if I can remember them. The first one is the pigs are in a closed colony, so you don't introduce any—

DR. VALDES: Well, you know, it's an international company built in Mexico in the north of Mexico. They cover all the international regulations, and they are in a desert in Sonora, which has five kilometers around with no animals. I mean, the regulation is really strict. I'm becoming mad when I visit them because I have to wash and change the clothes and wash the hands and many things.

DR. ALLAN: Okay. The other question is a little bit more general, is if there are knockout pigs being produced now, would it make more sense that if you're going to do any more islet T-cell transplants, that you use islets from knockout animals rather than from ones that are expressing alpha-Gal?

DR. VALDES: No, but the islets and the Sertoli cells are Gal negative.

DR. ALLAN: But the islets cells are—

DR. VALDES: Islets and Sertoli are Gal negative. That's the work done by David Cooper.

DR. VANDERPOOL: Well, Dr. Valdes and White, one of our hopes has been to maintain contact and communication with people who are doing xenotransplants in other places around the globe, and you're the first for us, and we thank you for coming. We do indeed hope that you continue to update us with the results of your work, and by all means, of course you have good contact through Dr. Groesch in our office, and we hope to hear from you further. And if at some point one or more members of our committee can visit your shop, I hope that could also occur.

Now let's take a break, just a brief break, and come back—

RAFAEL VALDES: Sorry. Mr. Chairman, something that I would like to share with you. This year, at the end of this year we'll get the first workshop on xenotransplantation in Cancun, Mexico. So it's December, I know that the weather here is awful, so it's Cancun, you can visit the Mayan ruins and I will keep you informed for all of you that are interested in our work. Thank you very much.

DR. VANDERPOOL: Thank you. We always appreciate a few extra benefits.

Now let's take a brief break and come back so we can focus on the State of the Science report. What we will do is review the main concerns the committee has with respect to this report and go as far as we can.

Also transportation needs, those of us who need transportation needs, be sure to check with the appropriate people at the desk who may also come in here. Thanks.

<BREAK>

DR. VANDERPOOL: Most of our committee is here, and a number of the ex officio members, so let's begin with an overview of the report, State of the Science in Xenotransplantation, by one of the co-chairs of the subgroup, Dr. John Allan.

Agenda Item: Overview of Draft Report on the State of the Science in Xenotransplantation

DR. ALLAN: Thanks. I'm not going to spend much time because we have a lot of questions we need to run through, and I know a lot of people are going to have to catch flights earlier than we had anticipated. Essentially just as a brief overview, this State of the Science report was a result of two working groups, one chaired by Megan Sykes and one chaired by myself. She chaired the science, basically the immunology and the overview of the potential impact of xenotransplantation, the types of procedures, the source animals and the products, and the major challenges posed by immunologic and physiological incompatibilities, and strategies to address those. The section that I was charged with is mainly infectious disease risks associated with animal-to-human transplantation.

And so it's essentially broken up into a background where we discuss the potential impact of xenotransplantation on disease, the types of xenotransplantation products, potential xenograft source animals. So really a background on what the challenges are with xenotransplantation, what the need is.

And then the second section is "Scientific Challenges in Xenotransplantation" and the immunologic rejection processes, which I think is very thorough, the physiological issues which generally don't get a lot of attention and haven't had as much exploration in terms of research, current approaches to xenotransplantation challenges, which more people are familiar with.

The second section really is infectious disease risks, and there's several areas that we focused on; general properties of infectious diseases that you need to think about in terms of xenotransplantation, maybe differences in risk assessment for primates—non-human primates versus porcine versus fish versus other source animals. Other sources of xenotransplantation products. We came back to viral persistence, latency, and species-specific virulence, because there are issues that are unique to xenotransplantation in introducing an organ into humans that you have to think about the types of viruses that might be able to find their way into humans and persist.

We also covered areas to sort of give an overview of what studies have been done to address the risk of PERV in particular in humans, and in the development of animal models to assess risk of PERV, and then the potential for xenogeneic infections other than PERV infections in humans. And then we also tried to tackle the control of infectious disease risks.

And this was much more at the forefront, I would say, a year or two ago. It still is. "Xenotourism,: An Emerging Global Public Health Concern." We have a lot of questions and comments, and we'll get to those hopefully today. Maybe not. We may have to come back for this.

"Knowledge gaps and resource limitations, molecular incompatibilities between species, types of animal models, sharing of resources, and funding issues. We have a lot to get through. And also what we covered, parallel or alternate strategies; in other words, xenotransplantation is not the whole thing, it's not the only alternative. We really wanted to provide as a comparison to other technologies and other treatment modalities that are being put forward.

And then last are "Recommendations," and I think that's something else we need to go through individually. And I actually have done that in one respect, where I go through each one of them.

So I think what I'm going to do is stop there, and then I'm going to turn it over to Harold and let him begin to go through each one of these questions we've been given and we'll see how far we get. Okay?

Agenda Item: Plenary Discussion of Draft Report on the State of the Science in Xenotransplantation

DR. VANDERPOOL: Great. At one point I thought we should just go through the several, all together, 15 pages of comments one after the other, but I think our time is too short and too valuable. As John said, we'll go as far as we can go with as much as we can say, and hopefully we'll try to exercise closure. If we need to, any of you here suggest why don't we call for a vote on that, or why don't we call for a consensus or something, so we'll do as much as we can in terms of establishing some parameters for what we are suggesting the subgroup should do with this in its draft to follow.

Rather than going through each point in these pages, I thought probably the best thing of all would be for us to go around the room, and just with each person here—we may not get around the room—both on the committee and ex officio—and see if you have a particular question or area that is of concern to you that you would like to raise to the Committee. And hopefully by doing that, we will get to the most essential issues we can think of.

Dan Salomon wanted me to leave you with his message— he's already left for California—which is he pretty much likes what's there, he likes even what's in the xenotourism section. And sure, a few sentences can be changed, a few things can be cleaned up a little bit, but he stands where he stands, open to suggested re visions. So I communicate that to you. Also Anthony Lubiniecki has given us a letter with some of his concerns—Bob, I'm sorry. Bob Mendez. And so we do have several places to begin.

I will introduce one point, and then let's just go to the left, beginning with Dick Kaslow. But my one question is that as I read this report and I see several comments—and I could point to at least four comments in the general comment pages—it's somewhat unclear as to what the Committee is taking to be the state of the science. What is the state of the science? Is it in its infancy? Now, that metaphor of infancy is used on page one, line four of the paper. If it's in its infancy, then it's just barely begun, and there are indeed sections in the report that would suggest that xeno is in—the science of xeno is in its infancy, particularly in this very strong section on what all the gaps are with respect to physiological compatibilities.

On the other hand, the other side of this report is, well, we have many challenges ahead and some gaps to be filled, but we've come a long way. And I could give a whole list of quotes from page 3, 10, and 25 in the report that suggest that scenario. With each of those views, oh, we're just in the infancy, there are a number of references and points that would say we're just in infancy; with others there are references to say, hey, we're encouraged, we've come quite a way.

Now, so my question is, can we choose a metaphor or can we choose a point of view that will summarize where we are, and whatever we choose, to be forthright about it. I can see how this report would say, well, xenotransplantation has come a very long way in the last 10 years—and by the way, I see this either as introductory or as conclusion. Come a long way in the last decade, and then bam, bam, bam, bam, these are harvested from the body of the report. And yet, there are many things to do. And I'm not sure where you say there are many things we have to do in order to move these to clinical trials. That seems to be a somewhat separate question.

But do you see my point? My question is, we're talking about the state of the science, and yet when you get into the body of the text, the state of the science means a lot of things, infancy, we've come a very long way, we're encouraged, we've got to fill gaps. But I think what we need is a clear and fuller discussion, more clarity about where we're going, or where the report is going and what's it saying.

Do you have comments about that? As I said, all of our points, I don't want us to get stuck with them, but comments about that lack of clarity. Because I think I see a lack of clarity in part because there have been varied responses to the report, depending on which side of this equation one is inclined to agree with. Oh, we're in infancy; therefore, let's don't put any funding in it, let's don't have partnerships. Or hey, we've come a long way so it needs money, it needs—see what I mean?

I think the puzzlement over what the report is saying and the disagreements over what it may be saying I think maybe caused you the internal way the state of the science is described; on one hand, only beginning, on the other hand, quite far along. Megan?

DR. SYKES: Well, I think you've got it right. I think we've come a long way towards solving the problems that have been identified, and the whole field has been focused on specific problems one by one; first, hyperacute rejection, then delayed xenograft rejection. When Gal was discovered, ways of overcoming the anti-Gal response were sought and they were found. And we've come a very long way, but yes, the field is in its infancy. We haven't achieved survival more than three months or so, and only of certain grafts, and some in heterotopic locations, and we don't know about physiologic incompatibilities.

And I think that those two messages are absolutely the ones that should be given from the report. I think that the message that xenotransplantation has a lot of potential, but realistically we think a lot of research is going to be needed before that potential is met, is the right message. And I think it would be a mistake to be unrealistically simplistic and optimistic and say routine clinical xenotransplantation is just around the corner. That sort of claim has been, in part, I think, responsible for the withdrawal of a lot of industrial funding.

DR. VANDERPOOL: Excellent point. My single suggestion, therefore, would be to say that right up front so that people don't sort of hear it now and then they're uncertain. They don't know what all is being said. One page says this, the other page says that. I think to say it in that forceful, clear way, and let the text then reflect that richness would make it a more powerful and more centered essay.

DR. KASLOW: Part of the problem that we might—that might help clarify it if we clear it up ourselves is that we're talking about the knowledge that's required, the science, and we're talking about this as a tool, a technique, an intervention. And so we could have come a long way with the science, and not gone very far with the actual implementation, the practical use of it as a therapy.

So if we could get that distinction across, it wouldn't solve the whole problem, because there's still—we've come a long way with some science and not with other parts of it. But at least if we knew that some of them are more practical issues that can only a wait the real practical interventions and the trials and so on, we won't learn anything until we can go into trial. With regard to other things, we can learn from the science and the laboratory work that's going on.

DR. VANDERPOOL: Are we taking notes? Is this all being recorded? Okay. Good. So those two distinctions, those two distinctions, is, one, a clear, up-front statement of where we're going, and then within that, the distinction between science and clinical applicability, I think would both strengthen the state of the science description you're making.

DR. ALLAN: So I think what you're saying is, is you want a clearer introduction as to what the heck this is all supposed to mean.

DR. VANDERPOOL: Yes.

DR. ALLAN: Because when we wrote it, there was two different subgroups and we sort of wrote each of our little subgroups, and we didn't really have a sense of what direction we were headed. And this is what came from it, and I guess what you're saying is, is that it really needs to be tied together through concise intro, a more concise intro?

DR. VANDERPOOL: Exactly. And as Meg said, not simplistically, but pull your assumptions right out and state them, and then as you go through, you make the case for those assumptions as you go through, and you may want a last paragraph that brings it together.

Okay. That's my one concern. I think there's probably a bit of a consensus in the committee on that. Robyn, you weren't here when I said we were going to go left and each person was to state his or her major concern. And you can say, I don't want this football, I pass it to Dick.

MS. SHAPIRO: Right. I don't want this football. I pass it to Dick.

DR. KASLOW: I don't have any overriding concern that I think I'm burning to hear addressed here. So I think if others do, they should proceed.

DR. VANDERPOOL: If you don't have an overriding concern, if you have something that you think is an essential issue that you would like clarified or something like that, feel free to give that. We don't have to give some more grand point, but yes, I think if you have a particular issue that you saw as a problem, then feel free to do that, too. And you may not have that either, Dick. So...

DR. SWINDLE: Yeah, I just want to support the idea that we should make a distinction between the scientific development and therapeutic development. I think that was an excellent way to put it. Because I think we're just a whole age a way from whole organ transplant that's physiologically functional for a long period of time, but it may not be so far a way in some of the cellular things. And I think progress has been made from—I've been on I think every xenotransplant committee since the first one in '95, and certainly there's a substantial difference in looking at the pathogens now versus what people were looking at 10 years ago.

So I think progress has been made, and I think that should be the opening paragraph in this. Other than that, I read through all the comments, read through. I still stand by the science as written, and the recommendations, and I think my interchange is to sort through those comments can be made.

DR. VANDERPOOL: Excellent point. I just want to say about this opening paragraph, to be specific about what Dick and Mike and I are saying, in that first paragraph, the second sentence, "What sets it a part from most other areas of clinical research and raises it to a level of special interest and concern is the potential (and unquantified) public health risk." Now, oh, my god, don't mention that yet. I mean, what I thought set it apart was its potential to meet critical health care needs and its potential health care risks. But certainly, if you flag in the very first question, look, the major thing here that's really unique is the potential health care risks, then I think you sort of rob the paragraphs that follow about what all it can conceivably do for health from their power.

So I think the intro is critical. Think through that. I agree with Mike on that very much. Okay. Karren?

MS. KING: I don't have anything other than the comments. In general it's very good.

DR. VANDERPOOL: Okay. Brad?

DR. COLLINS: This obviously took a lot of work and it's a nice job. That table on page three, these are just nitpicking things, the fourth bullet at the very—the last sentence about cystic fibrosis, I don't think a lot of kids are transplanted for that, so that just might be changed to adults. I can't think of—mostly adults get lung transplants. Okay. I understand your point, Marian.

And then the very last bullet point, we could update that to 2003 data, at least for those who died on the waiting list, and we could get the number of people waiting on the list as current as today. So it could be more current than 2001 for that.

And then at the very end, I was just reading Bob's discussion here. I haven't been able to get through all of this, but it sounds like there was some talk about artificial organs. That section could be maybe expanded just a little bit.

Those are my only comments. It's a great—obviously a lot of work and a great job by the science group.

DR. GROESCH: Has anybody joined us?

DR. MENDEZ: I did, Mary.

DR. GROESCH: Oh, great. Manny, could you activate the—

DR. LUBINIECKI: Yeah, this is Tony Lubiniecki as well.

DR. GROESCH: Oh, wonderful. So we have Dr. Lubiniecki and Dr. Mendez by telephone.

DR. MENDEZ: Actually, I've been on for quite a while. I enjoyed those discussions this morning.

DR. GROESCH: I knew that we were having some technical difficulties, so I'm glad that you were both able to make it through.

DR. VANDERPOOL: Welcome to both of you. Feel free to interject, interrupt as we go along. Right now we're going around the room to see what some of our major concerns or single issues we have in the State of the Science report, and so in turn, we'll turn to you for those comments you might have, also.

But at this point, let's keep going around the room. And Marian?

DR. MICHAELS: I have just some general comments at different points trying to address some of the comments that were made actually by the reviewers. And so I think it might be easier to just sort of keep with the introduction where you have been leading us so well.

And Alan, who is next, I guess—I don't know if Eda is going to comment, too—but I think Alan's comments about trying to have a little bit more in the introduction to just at least mention the concept that we didn't talk about prevention of some of the types of diseases that could be prevented, that we should actually address that, especially while we're addressing other potential answers to some of these diseases.

But I think rather than jumping back and forth, maybe I'll just come back in on different sections.

DR. VANDERPOOL: Any comments from the Committee about any of these? Feel free to do so.

DR. SCHECKLER: How are you going to do this?

DR. VANDERPOOL: We're just going to keep going around. We'll get to you soon, Bill.

DR. SYKES: Can I just address that we ended up with alternatives actually as a separate section at the end, and at the beginning of putting this report together, we had had it, I think, in the introductory section, and had also had a section, as I recall, on prevention. And I'm not sure what happened to that, but I think if we're going to—

DR. SCHECKLER: It keeps being taken out, much to my chagrin.

DR. SYKES: I think we do need something on it, but it should not be in the introduction, it should be at the back now with the alternatives.

DR. MICHAELS: I'm fine with that.

DR. GROESCH: And I think perhaps it was taken out in response to some comments that came in, but it's good to discuss it with the whole group here and get a better sense of it. I think that it sounds like people want to have it in. And Bill, we can work with you to get back the language that we originally had.

DR. VANDERPOOL: Okay. Among other really excellent comments were several by Eda and Dan and his group. If you want to attend to those, fine. I think several of those could probably be taken to heart without question, and fairly simply. But please tell us what you think. Eda?

DR. BLOOM: The comment that I made in the introductory statement was just probably a comment on the ability to misinterpret what was written. It wasn't a big deal.

And as far as the overall document is concerned, I think that the comments that you've received all raise very notable points. And I think that the document is good, but I think the document will be helped by all the input. I have no specific comments other than that.

DR. VANDERPOOL: Yes, I think the committee members I've talked to have taken the 14 pages to heart and see them as very constructive. A number of the comments, not all. Ellen?

DR. GADBOIS: I have nothing technical to add to this. My view is that you're here to tell us what to do, and so I don't want to feed you things to give back to us.

DR. VANDERPOOL: Thanks, Ellen. Tom?

DR. SPIRA: I'm also going to pass at this time.

DR. VANDERPOOL: Dan?

DR. ROTROSEN: Let me just start by saying I agree with all of the comments here that this is a very ambitious document, and I think it's coming together quite well. We actually had many more comments that I think most of you saw and were summarized in this table, and I think the science editor did a very good job of addressing those. And I think we'll be able to address any of the remaining ones probably outside of this forum. There are not major issues left, I think, except for one, which was commented on by a number of people, that sometimes the document has very much of a slant towards increased funding, and in some cases in specific areas, and that could be, for one, perceived as self-serving by some of the members of the committee; and second, could be conceived as beyond the charter of the committee in general.

And I would actually like to hear Ellen's comments on that. And as I reviewed the charter, on tab two, there are a number of—five or six bullets that describe the activities of the Committee, and none of them specifically address the need for recommendations on funding.

So I think it's important for you to speak on the Department's behalf, and Harold and Mary to determine exactly how much of an effort should go into addressing these concerns about funding, or whether they should be in the report at all.

DR. VANDERPOOL: Dan, at last count I put a dollar sign in the margins every time funding was mentioned, and in three pages, I had nine dollar signs. I thought it was way too repetitive. And I thought we needed to talk about funding, whether we should ask for funding, or whether we should talk in terms of—in more general terms, some of the language given by John in his almost full page of text about having joint partnerships and needing to have joint research endeavors.

I think you made a number of good points. One is that we need to be careful in our criticisms of government agencies, because even as we saw today regarding the availability of closed colony animals, a lot is being done. So maybe—I mean, what should we say? I know in my comment, and these never made it to the document, I think in part because I was supposed to facilitate and it would be better not for me to have any comments in there, was that it would be better to bring those recommendations in to a rather discrete final paragraph, and sort of pull them together about these things can't be accomplished without building partnerships between universities and government and industry.

I know Mary did some checking about whether we could recommend these kinds of things, and what was it you discovered, Mary? She did this on behalf of me.

DR. GROESCH: Well, a couple of the reviewers had mentioned that they thought it could be considered a financial conflict of interest to have a recommendation about increased funding. And I did consult with our ethics, the NIH ethics office, on this, and I spoke with Holly Beckerman-Jaffe, who has given us conflict of interest training previously, and also Gretchen Weaver, who is the NIH ethics counsel. And I have a statement that it will just take a moment to read it, and I can give you copies of it, but it addresses this concern about whether it's a conflict of interest.

And it says, "Some members of the Secretary's Advisory Committee on Xenotransplantation conduct research involving xenotransplantation, and through their employers they may apply for and/or receive federal support. Questions may arise as to whether those members have a conflict of interest if they participate in discussions or recommendations regarding federal support of xenotransplantation research. For conflict of interest purposes, such discussions and recommendations are considered to be matters of general applicability that do not provide an advantage to a particular institution or individual conducting xenotransplantation research; rather, a recommendation

for more federal funding for xenotransplantation research would affect all recipients or potential recipients equally. A general matter poses less risk of a conflict of interest than a specific party matter."

And what is meant by that is, for example, if the Committee were making recommendations regarding the funding of a xeno research project that's only conducted or could be conducted at a particular institution.

"Thus, HHS has granted general matter waivers under 18 USC 208B to committee members with such potentially conflicting interests. In granting those waivers, HHS certified that the need for the committee member's services outweighs the potential conflict of interest created by their financial interests."

That's the statement. And I think the bottom line is that a general recommendation would not be considered problematic, but if it were so specific that it would not apply to all researchers, then that would be considered problematic.

DR. ROTROSEN: I don't see anything in the report that that's specific. I think the question is whether the charter of the Committee even calls for recommendations on funding, and I think the general enthusiasm that comes across in the report, it will be implicit from that that there's a need for additional funding that could be stated, but I don't think it needs to be stated so many times as it currently is. That's what gives it that flavor of self-serving.

DR. VANDERPOOL: That was my point, Dan, that the repetition is part of the—begins to—you begin to get pressure. It's like someone doesn't ask you would you like to buy something, but you feel like they keep stopping you on the street and asking you again.

And so I do think maybe implicit to our charge, advise the Department on the current state of knowledge regarding xeno, "on the current state" might be just what it is right now, but you could take that charge to be, okay, the current state is this; this is where we need to go, and we can't get there without that. That is stretching it a bit. Robyn, who has also got the eye of a lawyer, even as this letter demonstrates—my son is a lawyer, so some of my favorite people are lawyers, including Robyn. So Robyn, do you have a voice on the charge?

MS. SHAPIRO: I'm not concerned about whether the charge could be interpreted so as to include mention of this issue. I mean, not only what Harold just said, but the last bullet that is discussed, blah, blah, blah, including socioeconomic issues. This certainly could be a socioeconomic issue.

And even the one above, advise on policies, well, they refer to the guidance, but policies, more generically speaking, could be funding policies. So I'm not terribly concerned about that.

DR. ALLAN: Harold, more basically, a lot of the recommendations that are in this report have not been significantly vetted even amongst the committee. So I think it would be of interest not to assume that everyone believes that throw more money at it and it should be interpreted this way. I think it's up for—and it's more discussable in terms of how this thing comes across when you go through the bullets in the recommendations. We never sat down and said, well, I like this one, I don't like this one, we should change this. We haven't done that. So this is a good forum to even discuss how you want to say it.

And I actually thought that what you had written in there, you said more general, and you even gave a little—you gave some suggestions, I thought those were very good in terms of how to say it, rather than case in point, I showed Megan, which was on page 27 where it says, "The SACX considers it risky to depend on industry, non-profit organizations, or academia to assume a disproportionate share of the financial burden." That's an example where we could just generalize that instead of have something that Harold says is a dollar sign.

DR. SYKES: I agree. I mean, I think that that word does come up and it's too obvious. I think, though, that there are some problems that we try to identify in the science that won't get solved without more resources, and that's probably a nicer word to be using, and I think that the field is particularly at risk right now because of the withdrawal of industrial support that's been quite widespread.

And I think those two things should come across, and I don't think that there's anything that—I think those two conclusions are well supported by what's in the report, and I think—I agree that some of the wording does need to

be changed even at this point. It has—I think the last draft did incorporate a lot of those suggestions, but there's still more to be done on that.

DR. VANDERPOOL: Alan?

MR. BERGER: I would like to add one more thing to it, that there's actually the assumption made that private industry are making short-term decisions, and that's not always true. I think we've gone—or this committee has gone too heavy. I mean, if private industry is not putting money in it, I think there are deeper concerns there that we should take heed of in this report. I don't think you can just write that off in that private industry are short-term thinkers. In my own experience in working in private industry for many years, if there's money to be made, they're in it. And so I would be careful to write that off in short-term thinking.

DR. VANDERPOOL: Excellent point. A lot of private industries seed their business for years at significant debt before they finally see the promise. Thanks, Dan. Laura St. Martin?

DR. ST. MARTIN: I just wanted to mention that given the scope of potential use of the xenotransplantation products, the text on page three seems to overemphasize the use in treating diabetes with porcine islet transplants. We have not yet maximized the use of human pancreata for islet transplants, and there are some limitations that have little to do with a shortage of pancreata. There's really an underutilization of some of the pancreata that are available.

And I would suggest that if you are going to add something about prevention to the alternative therapies, to perhaps add some mention about exploring or stating methods to increase consent to donation and to increase procurement and utilization of organs from consented donors.

DR. VANDERPOOL: Thank you. Responses? Okay. Glen Drew?

MR. DREW: Nothing specific, just an observation that as the review of papers this morning demonstrates, it's a difficult task to describe the state of science because you're shooting at a moving target, which is merely a challenge for the committee.

DR. VANDERPOOL: Indeed. We're trying to take a snapshot at the NASCAR racetrack, I suppose. But I think that's one of the terrific things about this report, that we can state something that can educate the public about where we are, even though by the time it's actually released, there will be probably two or three announcements that have already occurred that says we're further a long. Okay. Good point. Alan?

MR. BERGER: I will try and keep it to probably three points for now. The first one, when this committee was set up, it was set up so that there would be some representation on animal issues, which have actually never been discussed since we've been here. So I would like to make a few points that I would love to see addressed in here.

Something on ethics. I'm not naive enough to think that we'll get into a broader picture of animals in society; on the other hand, there are things about using animals in research, and some things that are very particular to xenotransplantation that I do think that should be mentioned in this report and should certainly be discussed in this committee sometime in the future.

You know, the first thing that really strikes me is that animal-to-animal transplants have been going on for 40 or 50 years, and we've been killing thousands and thousands and thousands of animals over the years, and it really needs to be discussed in terms of the benefit of actually doing that.

Secondly, when we talk about animal husbandry, we're talking about the care of animals based on humans, not on the animals themselves. We're not looking at growing up in closed colonies for really a short period of time, whether that's really beneficial for the animals themselves. It's been brought up that we've talked about cloned animals, where when we're cloning pigs, for instance, many of those animals die after birth, or many of them show up with deformities.

And probably last of all in terms of dealing with animal issues, when we have an animal recipient, the animals may in fact be kept a live to the very end, where in maybe even other research projects, they might be euthanized at an earlier stage just to end suffering, or in a more humane fashion. This type of research may actually call for the animals to be kept alive longer, and I'm definitely open for comments on that. But in a very specific sense, I would really like to see us put something in this report that has to do with animal welfare issues.

Secondly, I was just curious, Brad had mentioned this little table on page three, and again, someone can answer this one. The very top one, "Congestive heart failure affects 4.8 million Americans, half of whom, unless they receive a heart transplant, will die within five years of diagnosis," which would tell me that we should have 2.4 million people sitting on the heart transplant list, of which there were 6,990. So either that's an overstatement of the number of people that could use heart transplants, and I'm just curious about are we overselling with that particular statement.

And last, which anyone can comment on, obviously, this has been asked before, but I'm very curious, and to some of the experts that might be here, when we are genetically altering animals, and we have plenty of experience with breeding animals, whether they be farm animals or dogs and cats, then when we're genetically altering an animal, and in particular through breeding, we're changing the characteristics. And there is a cause and effect, where we're dealing with PERV and some other characteristics. If we change that, are we causing more potential problems that we really don't know about, and is that something that anyone else on the committee is worried about besides me, that somewhere down the line, our genetic altering will cause something that might be extremely negative. Thanks.

DR. ALLAN: Do you mean altering in terms of the animals or the humans?

MR. BERGER: Well, the animals that may alter the humans once you actually do a transplant. If you're removing PERV or Gal or screening for any other kinds of diseases, and you're breeding a new animal, for instance, and you're changing some characteristics. And we've had this—somebody presented a talk a year and a half ago that there is a cause and effect, and I'm just curious about what that effect might actually be. And it's certainly an unknown, from what I can understand.

DR. ALLAN: It's much larger in the biotech area, the ag biotech, where they're cloning and introducing genes for food. So that's going to be much greater than this, but it doesn't diminish your point.

MR. BERGER: Right.

DR. VANDERPOOL: I think, Alan, you definitely have a point about how we have not really dealt with animal issues in any kind of a sustained way, not to speak of even one or two addresses. I think an asterisk note is not enough. But actually, as I read both of these papers, they are select topics, and what goes with those topics.

One of the things we did on page two of the Informed Consent document was say that right up front, there are a lot of ethical and legal and other issues, and we say, for example, the risk of introducing infectious disease in the public, well, that's dealt with. The naturalness or unnaturalness of transplants from non-human animals to humans, the genetic manipulation and the use of non-human animals. That's a topic. We have not really dealt with it.

And so the question would be, should we in some way give that topic—does this topic call in to play that topic, and if it does, then we ought to mention it. If it doesn't, then this is one of those topics we have unfortunately not dealt with in detail, nor have we dealt, except just in passing today—not in passing, but for a very brief time today with international concerns.

But I hear you, and I'm not sure that this is the place where we want to talk about animal issues unless we want to say that they're at some point—with respect to genetic manipulation, that there are some concerns over this, and I don't know what the science of those concerns would be on the effect of the animals and on the degrees to which these manipulations would cause unnatural, strange things to happen in nature. I don't know. Michael, do you have—

DR. SWINDLE: Yeah, actually, I asked some questions when we had the knockout people here before, and in fact everybody has said that, yeah, we're getting unexpected mutations that show up in some percentage of these animals. And I forget what the percentage is, but that happens when you do transgenic and knockout manipulations

in every species it's been done in. And so they are coming up with mutant animals in a smaller percentage that's not what they expect to develop.

Now, whether that has deleterious effects upon humans after you implant tissues from them, I don't think there's any science to go one way or the other on that as far as whether using animals in research, period, or using these donors or a whole separate—I mean we could spend every meeting we had just on the ethical issues from that. He's correct, there's nothing in here about the ethics of that, except you gave a short presentation on it right at the first meeting or the second meeting?

MR. BERGER: I actually didn't.

DR. VANDERPOOL: Lilly did.

DR. SWINDLE: Lilly, yeah. And other than that, we haven't really done anything on ethics. I don't know what you would say, having not had these addressed, other than to say it in the generic fashion that you just did, that unforeseen things may happen and there are ethical issues and societal issues with this, and make a general statement that it's out there. But I don't think you're in any way prepared to make a definitive statement about the ethics of xenotransplant based upon this committee's activities over the last couple of years.

DR. VANDERPOOL: Robyn and then Ellen.

MS. SHAPIRO: I agree with you, Michael, and I go further. This report is a report on the state of the science, so if there's something about these animals that have a scientific message, then we should include that. But the ethics of animal research is a whole other topic which maybe this committee should look at in the future, but we chose not to. I mean, it's just kind of how it was. We picked Informed Consent and the State of the Science.

DR. ROTROSEN: But we did that because I think there's nothing that's come out in the discussions or in what literature there is that there's something unique about the—from the animal's perspective in xenotransplantation research versus any other field where animals are used in research.

The example you alluded to earlier, maybe these animals are being kept longer and not euthanized, well, that's not true. In allotransplantation animal models, we keep them as long as we can, in many cases. And you heard this morning from David Cooper and others, I think, that some of their animals were euthanized using ethical principles that are not unique to xenotransplantation.

DR. VANDERPOOL: Actually, the regulatory structure is a concern for the ethical integrity of animal research and how they're housed and how they're kept and so on. That's supposed to be one of the foundations upon which xenotransplantation is also predicated. We haven't scrutinized those regulations, and—

DR. ROTROSEN: I think maybe a good compromise would be to make very brief mention of some of these principles, and refer the reader to a publication on ethical treatment of animals in research and leave it at that.

DR. VANDERPOOL: But Dan, I think—I mean, I would side with Robyn in terms of that's not the purpose of these two reports, and I think we need to stay with our purpose. But I think a number of our comments are saying we recognize that this has not been an interest we've had or a focus we've had or something we've addressed, and it's certainly one of those topics that has called for more attention than we've given it. Marian, Ellen, and Michael.

DR. MICHAELS: I actually like the idea of adding in a statement here and there at different parts of the scientific area commenting about the fact that IACUC's have to be involved, and that there is appropriate treatment in the United States in different documents for the appropriate care of animals used in studies.

We are stating very clearly in here that we think animal models are actually an important part of moving this science forward, and also what is happening currently. And so I don't have a problem with putting in a comment about that, even though that wasn't the major focus. So I think Dan's compromise is actually one that I would strongly entertain.

DR. SWINDLE: On page 22 of the report is some language that I put in that has been modified that does refer to the idea that people who are doing xenotransplant ought to be AAALAC accredited, which is the highest standard you can have. It also refers to the various laws, regulations which have housing, husbandry, and ethical review guidelines on it.

So I would say to you that in that brief form, that it already alluded—this report already alludes to the fact that there are standards and you've got to follow them. It's also in the FDA report as well.

DR. VANDERPOOL: Ellen?

DR. GADBOIS: One course of action you might want to consider which some other advisory groups have done as they're either wrapping up their work or have significant change in membership coming up, as this group does, is to produce a short document just indicating issues that you felt that you didn't address, but that the next group or set of people may want to pick up on and explore further. It's a nice way to wrap up what you are doing, but show there are some things that we didn't get into that the next crew may want to consider.

DR. SYKES: Harold, just one more point on that. I was going to suggest that since we do have really two separate documents, but we were asked for a report, we might just start the whole report with a preface that indicates why we've chosen these two topics and that we've left certain others out, and specifically refer to the animal issues. And as Ellen just suggested, suggest that these could be addressed by a future group.

I also wanted to address one of the other things that Alan brought up, which was the question of why there are so many fewer people on the transplant waiting list than there are with, example, end-stage congestive heart failure. And there is a good reason why the transplant list really is just the tip of the iceberg, and that's because so many people, many people with end-stage organ failure have other complicating organ failures. Particularly, congestive heart failure often leads to renal failure, liver failure, et cetera, et cetera, and those patients will be beyond being considered for transplantation.

So we could actually—the transplant list doesn't represent by any stretch all of the people who could benefit from the xenotransplant. If organs were available in unlimited numbers, we wouldn't have to just have the tip of the iceberg, we could catch people earlier before they get to that stage.

DR. VANDERPOOL: Let me see if we can get a sense of the meeting. When Michael was referring us to page 22, lines 14, 15, and so on, what's the sense of the meeting that one or two sentences could be added there to say that these regulations underscore the importance of ethical treatment for animals and maybe something else, and this is a topic that deserves considerable attention unto itself, something a long those lines?

DR. SWINDLE: Yeah, I think you can add to it fairly simply to make a general statement at the bottom of that to say that there are concerns about both the animals who are under research and animals who are being used potentially as donors, and the ethical concerns need to be considered in line with existing federal regulations.

DR. VANDERPOOL: Let's get a sense. How many in the group today would favor that this reference to the ethical use and care of animals should be mentioned at this point in the report? Let's just have a show of hands.

DR. SCHECKLER: I would mention it, but I think it's in totally the wrong spot. It's in the section that talks about control of infections. This is a much more generic statement, and it's more than just the control of infections, it's the use of animals. So either it deserves its own bullet as a recommendation, or it's mentioned further up front in the document. It's fine the way it's stated here, but this particular section on page 22 or such, control of the infectious disease risks, that's where it starts on page 20. That's where this is all located. And this is a much broader issue than that. Actually, I kind of agree with Dan and Marian.

DR. VANDERPOOL: Isn't there a separate section on?

MS. SHUMAN: I would make the suggestion of possibly including that where we talk about potential source animals.

DR. SCHECKLER: That's fine.

MS. SHUMAN: Which is on page five.

DR. SCHECKLER: That's good.

DR. VANDERPOOL: So the recommendation would be to move it up and find a place on page five. Good close reading there, Bill. Okay, Bill, you have—

DR. SCHECKLER: Oh, yeah.

DR. VANDERPOOL: I thought you would say no, I pass on Sharon.

DR. SCHECKLER: Not a chance, in part because Mary didn't find my e-mail with my comments and they aren't in the working book. So I have to say something, but first I would like to summarize our last six meetings, if I might, with a limerick, because I did this at HCPAC all the time.

DR. VANDERPOOL: I have a copy, but I couldn't bear to read it. No, go ahead, Bill.

DR. SCHECKLER: The name is "The Reports":

> There once was a group called the SACX
> Which struggled hard to collect all the facts.
> They talked and they wrote
> And brought their work to a vote
> To provide truth, well beyond any pretext.

So that's what we're trying to do here.

There's five short things, comments, generic comments that I want to make, and I will, as I did with this morning's report, give them all to you, Harold, so you have the marginal notes.

First I have to go back to the infectious disease risks. And I agree entirely on page one, paragraph one, that putting the third sentence up there, the way it's worded now is just the wrong message for the whole science article. As a matter of fact, I would like to suggest—maybe we can get a consensus on this between John and Richard and myself—that we ought to use the terms instead of "potential and unquantified public health risks," we ought to use the term "theoretical infectious disease risk," rather than making it as generic as public health risk, and making it related to the entire population. I think that is rhetorical, theoretical, hypothetical, and scientific overkill.

DR. VANDERPOOL: Bill, let's harvest your point as we go. How many are in favor of that linguistic change, "theoretical infectious disease risks" rather than "unquantifiable"—

DR. SCHECKLER: Rather than "potential and unquantified public health." It's a fundamental issue with the entire document.

Basically, the follow-up is that the infectious disease risks are principally to the xenotransplant recipient, and potentially, if you look at the retrovirus paradigm like AIDS, to the intimate contacts of the recipient. But as I tried to say this morning, I think beyond that, except possibly needle stick injuries to health care workers, the risks are virtually nonexistent.

DR. ALLAN: But see, I had this argument with you this morning. The thing is, is that any a gent will be transmitted that had a long clinical latency period to a patient. If that patient survives and goes back, and that's the goal of xenotransplantation, is to make the person healthy again, then they go back out into society and you get sexual or blood—sexual transmission, which has long clinical latency periods. So I would say that that's the major concern; it's not to the individual, it's to the society. And that's why we're grappling with this whole issue, just

simply because of that, of the possibility of transmitting a new emerging infectious disease. You can argue it's theoretical, because everything is theoretical until it happens. And so it depends—

DR. SCHECKLER: It's a hierarchal concern about risk. It's a relative risk and an absolute risk, and my suggestion is that the potential real, most likely risk is to the—I think I'm agreeing, to the patient, even though it's a long latent period, and to the intimate contacts. But not to the world.

DR. ALLAN: No, but that's exactly what the point of this, in dealing with infectious diseases, is that it is that risk that it gets transmitted from person to person to person over a long period of time. Let's say maybe it's 20 years. It could be a year, but it could be 20 years. Just like HTLV infection in humans, started in one individual and spread throughout the world.

DR. SCHECKLER: Harold, you asked for a vote. I'm perfectly willing to accept. I won't abstain. I'm willing to accept the view. I think we have a fundamentally different view on how important this is to highlight.

DR. VANDERPOOL: I think at this point that this is flagged as an issue that the subcommittee needs to iron out, because I don't see that voting on this is going to help one way or the other until you all can iron out the issues and decide what wording you would prefer and bring to us, the rest of us. I mean, and put it into a draft.

DR. KASLOW: Well, there's another simpler way to handle it potentially, and that is to say what has set it apart. Because that's where we started three years ago, five years ago, whatever. Where we are today in our thinking about it may be different, and we obviously still have some disagreement, but there's no question that we started out thinking, and that was what set it apart for us at the time.

DR. SYKES: Right. And I think what you're saying, Bill, is true that currently with our existing regulatory guidelines and our animal husbandry practices, it is a theoretical risk, and that we might want to temper this sentence. I mean, I think what it says is true in the broader context of we're worried about other countries not having regulations. I mean, it is true that if somebody was routinely doing xenotransplants from farm pigs, there would be a pretty good chance of having a new infection brought out and spread to other people.

So we might just temper the sentence with another one, saying that regulatory guidelines and advances in diagnosis and husbandry have gone a long way towards reducing these risks, or something like that. But I don't think we should take a way the concept that there is a public health risk there, because it's still very real in the context of many of the issues that we discussed, like xenotourism, for example.

DR. VANDERPOOL: Well, as I read through earlier, I think we agreed earlier the document will begin with a different couple of other paragraphs. What sets it apart to me is not merely infectious disease, I promise.

So I think these are issues that the subgroup needs to hash out in greater detail. Mary has said that if necessary, we may need to come back for another meeting in a fairly brief period of time in order to finalize this report.

DR. GROESCH: Or we could have a teleconference discussion.

DR. SCHECKLER: Teleconference. Let me get to the non-controversial parts of my thoughts. Those were the first two. The section six, and that's on the alternative therapies, "Parallel or Alternative Strategies," I think it's way too long, I think it goes into much too much detail, and I have two alternatives. One is delete it entirely, at which point you don't have to mention prevention, or include—shorten it up and include something about prevention a long the lines of the best alternative is to prevent the acquisition or progression of the chronic diseases that lead to end organ failure. Prevention activities need to be promoted by all available means, et cetera. I have the wording here. It's what I had tried to put in the introduction and it got taken out of the introduction, got reduced initially to one sentence, and then removed entirely, and now we have the threat of infections up there.

So I don't care which direction, but if you're going to mention these other alternatives which are highly theoretical and in the future, you ought to mention the epidemics of diabetes and things that we have right now, which is diabetes, you remember, and it's type 2 more than type 1 is the most common cause of end-stage renal degree.

DR. VANDERPOOL: Comments about Bill's point?

DR. MICHAELS: I would put forward that we put in some prevention, and also I think someone earlier had mentioned the concept of also trying to maximize using organ allocation, the organs from humans as well. So I think that should—

DR. VANDERPOOL: Yeah, Alan mentioned that at one point. So let's have a couple of other comments and see if we can reach a consensus on this.

DR. SWINDLE: I think it's important to leave the alternatives in there and add to it. And I do remember your sections as it was before, and there was something about increasing organ donors in one version of this. I think all that belongs back in, but I think because people will read this and say, well, why didn't these people consider artificial organs and things like that if you reduce it to one line. I would rather show that we did spend some time looking into the background of alternative therapies, we're a ware that they exist, they may or may not replace xenotransplant for the completeness of the science, and just add to it.

DR. ALLAN: They could just be cut down a bit.

DR. VANDERPOOL: I must have canceled everything by punching my purple button instead of my green button. We are having Mardi Gras in Galveston right now, and purple, green, and gold are confusing.

And so are we generally agreed that in this alternative section there be something on prevention as well as on increasing donation?

DR. SCHECKLER: Okay. I'll accept that.

DR. VANDERPOOL: Increasing donation, by the way, will be a good point for the DHHS, because that's one of its things. And we need to recognize that ourselves. Alan Berger has spoken about that several times. Is your list exhaustive?

DR. SCHECKLER: No, no. I have two brief points. Bob was trying to say something on the phone there?

DR. MENDEZ: Yes. Harold, I was just going to say that I think it's great to put something in about increasing organ donation; however, I think we have to be very somber about the fact that no matter what we do in trying to increase organ donation, that certain types of illnesses such as diabetes, we will never get even close to being able to provide the adequate needs of human organs, unless, of course, we start to again get into the cloning aspect of things.

DR. VANDERPOOL: Excellent point, Bob. I think for us to present changing organ donation, human organ donation patterns is the answer for the pressing medical needs is certainly a stretch. Thanks.

DR. SCHECKLER: All right. The xenotourism, I talked to Dan before he left, and he kind of likes the way he had written the pages that are currently pages 23 and 24. I suggested perhaps a shortened version, the Cliff Notes version, with a comment on the World Health Organization initiatives that Eda talked about this morning, and the State Department initiative that somebody mentioned also in terms of background to the whole issue of xenotourism. Because those things have happened since we started talking about this, and those are, actually, I consider major advancements that minimize some of the concerns that we had two years ago when we started to talk about this. I don't know how to wordsmith this, but it seems like it would be useful to include those. And my final point is—

DR. VANDERPOOL: Let's finish and close on that point. In talking to Dan at some length about this, Dan said what has already crossed over the Jordan is in the promised land with respect to the State Department's initiatives and so on. So the good that he foresaw in this section has already been accomplished.

So I think Dan, who pressed so much on this, would be generally favorable to cutting it down a little bit, taking Dan Rotrosen's comments about being discreet about who we mention and not appearing too critical to—I mean, we can

be critical if you wish to, but just by not naming individual entities as if we're pointing them out as inferior to what we're doing. I think that's all well taken, also.

Any other comments, quickly, on this point? Last one.

DR. SCHECKLER: My final point is a way out of the funding dilemma, how to get the dollar signs down to one or two instead of nine in your report. The way I—and you all that are researchers can correct me, but the way that I've always been taught to think about research is fundamental research, bench research, which the NIH is used to funding, which then leads to translational research, where you try to move things from the bench to the clinical arena and translate what you've learned in the basic sciences of things into something that's clinically applicable, and then in the final stage you have clinical applications of what you have learned and it becomes standard therapy. Like allograft renal transplant therapies right now are standard therapy.

So it seems like the government and private foundation funding of fundamental research has been traditional; translational research is collaborative research amongst private foundations, non-profit foundations, government, industry, and other resources, and the clinical applications is where things move much more towards the private sector, and then be paid for by our health care resources, which are inadequate right now. But anyway, that's like animal rights, that's a whole different issue.

But it seems to me that then put in that context, it kind of meets everybody's test as appropriate. And it's very clear to me that we've come a great distance in the three short years that we've been meeting in terms of what we understand about PERV, what we understand about possibilities for transplantation; the non-human primate data that we saw, even the data from Mexico City was quite interesting, is quite different than where we were when we started out with some of this, and it's liable to continue to go on, provided there's a source of collaboration. So that's the structure that I would suggest would be a useful structure for this.

DR. VANDERPOOL: Comments? Let's keep moving. Sharon?

DR. KIELY: Most of what I had to say related to the xenotourism. And I would just say that it is a brief section, and I agree with just about every comment that's here, so I won't say another word on that. But it did seem odd to me that it is a brief section, we've said now it's supposed to be a briefer section, and about three of the 14 recommendations relate to it.

And so particularly on number 13, following number 12, it's on page 31, that we're talking about communicating with the public about the risks and develop educational materials, and I think that might be a little premature, given that we haven't really defined the scope of the problem nor cited a lot of the anecdotal and other comments in the body of the paper.

And while I do agree with the sense of it, that there is some concern—and personally find it to be a significant concern—I do believe that some education needs to take place, but I'm not necessarily convinced it's to the public at this stage of the game. I don't know what we would say to the public, given what's in that section. And that's all I have to say.

DR. VANDERPOOL: I suspect these recommendations will need to be re visited after these changes are made. Good points. Jim?

MR. FINN: I've got a couple of points I would like to make. If you go back to the beginning, in the introduction to the document, this will be publicized soon, this will be on every register and available to the public. We could have covered in our summary sheet to the media, because the media love to get a hold of this kind of a story, they love a bittersweet story, and I think we should have a cover sheet ready for them, like a press release or something to that effect. I can envision—I've been involved with the media quite a bit, as you all know, and I can envision two stories coming out of this; "World Sees Great Hope Through Xenotransplantation" or "Death by Design, Xenotransplantation." You know, the media doesn't always say that's what they should be saying. I just think we should have some sort of a preemptive strike ready for them.

DR. VANDERPOOL: Well said. To the effect that this goes to the public, I hope the subcommittee also looks a little closer at some of the language used. I mean, I know it's wonderful to have the terminology at the back of the document; on the other hand, when a phrase like "closed colony" or "complement system" is used, I think it would be very helpful to give at that point a very brief definition, and then maybe in parentheses, "see glossary of terminology." I think that would make it more readable for many people in the media and many science type editors who will be interested in this. Okay. Megan? I don't know where John went.

DR. SYKES: I think he had to leave. Well, we've covered a lot of the things I was going to talk about. I found a number of just small inaccuracies and corrections that I could just hand in separately, and also I think we need to update some of the science that has taken place since this was originally written.

Xenotourism, I agree with toning down the specifics. I don't think it hurts to have a strong statement about the importance, even though the government is already a ware of that and these initiatives have been made. I think reinforcement in this report is a good thing.

The sharing of—the funding issues, I mean, I have a lot of specific places where I would like to make suggestions for further changes that I think we can tone that down.

My only real additional comments were on the recommendations. I found the wording of the recommendations a little bit strange. It wasn't really clear to me who we were recommending this to. I mean, obviously this is a report to the Secretary, but the Secretary isn't studying pigs. So recommendation one, "continue to study pigs as a suitable donor," I mean, we all know what we mean there, but it's just written in, I think, a strange way. And I think that the wording of all of the recommendations could be made more general so that they're more appropriate for a report like this.

There were some things that I found—oh, yeah, one suggestion. The last—the section on artificial organs, I wondered if it might be worth including a short discussion of the investment that has gone into the artificial heart program. I know that there was a very long and intense, I hate to use the word "government-funded" effort, to get to the point where we are now, and I don't know if that's a good thing or a bad thing to put in the report. But I think it is instructive, and it does serve to remind people that if you have an ambitious goal, it does require a long-term and, unfortunately, expensive effort. So I just would like to know what people's thoughts are about including something about that in there.

The other comments on the recommendations, number seven I found strange, "Build industrial-academic partnerships within which sharing of re agents and research animals is ensured." Well, I think that's unrealistic. How will that occur? If we're going to recommend something like that, we need to recommend a way to do that. And I don't have any good ideas, and I don't think we've come up with any yet, and that's why it is worded in this—I think, Dan, in your comments you wrote wishful thinking at some other point in the report, but I think that it's the same idea here. It just sounds like wishful thinking, with no specific means for achieving it.

And then recommendation number 10 I think that we should re word, because it also is really impossible, "Ensure that all treatments involving the use of xenotransplantation products in U.S. citizens comply with PHS"—so there, if we could just reword that to make it more realistic.

And then the number 12, I think we need to be a bit more inclusive in that recommendation, not to only discuss Americans leaving to go have a xenotransplant and then coming back, but also people who are coming here from other countries, who live in other countries and have had xenotransplants there. So the others are more stylistic suggestions.

MS. SHAPIRO: Now I want the football.

DR. MENDEZ: May I make a comment on that? This is Bob Mendez. I agree with Megan's suggestion that perhaps we place another sentence or two about the artificial organs, and I've faxed to Mary some of the—some references to that. But I spoke with Walter Debinski, who is the president of the International Artificial Organ Society, and these hybrid grafts and the LDATS that are used, John had mentioned in one of his comments was that we should perhaps put more in about the LDATS and where they are—or the AbioCors, where they're out two years

now. I spoke with Bud Frasier from the Texas Heart Institute, who had one of the grafts for the AbioCor, and he had done seven, and they don't look all as promising as we had hoped that they would be. The hybrid ones for jump grafts to re start cardiac function looks good, but as a long-term cure or a substitute for human or xenograft, I don't think that—we can say that there are ongoing studies, but boy, the money they're pouring into it is not really demonstrating a tremendous advance in that field yet.

DR. VANDERPOOL: Thanks, Bob.

DR. SYKES: Bob, do you think it would be therefore unadvisable to discuss the effort that has gone into development of the artificial heart?

DR. MENDEZ: No, I think we can mention it. I think we should mention it. But I think we should put it in the context that its availability and benefit on a long-term basis is far from—is every bit as more distant than xenograft or any other types of therapies might be.

Now, the hybrid organ for temporary resuscitation of the organs, that's going to be—that's something else. And that is succeeding very nicely, and it will perhaps cut dramatically in the number of organs that do go to end stage. I wouldn't cut it out completely, but I wouldn't be too enthusiastic about it being a great alternative.

DR. VANDERPOOL: Bob, you're going to send statements that—well, we have your statements here, but also references to that effect. As you recall, early in the report there is a reminder that hearts are more than just pumps and kidneys more than just filter, and I know one of the people working for BioMed, and it seems to me that's a real flaw of those studies, just to assume that these are mechanical—a heart is a mechanical instrument.

So I think putting in some words to the effect that you've mentioned about the guarded optimism, or even to mention some of the apparent problems, it strikes me that the public probably has greater expectation of how—you know, *Time* magazine is going to have an artificial heart on the cover any day now, and it's going to be the savior of the heart patient. It's probably just a popular myth that deserves some mention on our part. Thanks, Bob.

MS. SHAPIRO: Recommendation number nine, I think, is either something we don't want to include or we want to modify, "Develop approaches to protect commercial entities against broad liability for the consequences of possible zoonotic infections." Not clear which commercial entities we're talking about, and clearly if we're coming out with all these recommendations about how to be safe, and they're going to violate them, we don't want to protect them. So I would not get into this myself.

DR. VANDERPOOL: I agree. And Dan made an excellent point. If there's any single sentence that could appear to be not merely self-serving, but also represents conflicts of interest for certain people on this committee—not to speak of our friends—this would be it. So I would be for just taking that one out.

Okay. Now, do we have a sense of whether that number nine should be kept or changed or taken out? Those in favor of deleting it, raise your hand. Those in favor of keeping it, raise your hand.

DR. SYKES: Can I just discuss it a bit more?

DR. VANDERPOOL: Sure. Megan has a wonderful alternative. Let's discuss it more.

DR. SYKES: I'm sorry, but we did put that one in for a reason, which is that liability issues have been one of the factors that have led to withdrawal of industrial support. And by the way, I don't have any stock in any biotech company. So it has been a real impediment, and I'm not sure that that will ever be overcome without some creative new strategy for indemnity about that.

DR. ROTROSEN: Harold, can I comment on that? I think you're right, it's a major impediment, but the only—or probably the best precedent I know of for an indemnification program is the vaccine indemnification program. And there you've got a tremendous societal benefit to people being vaccinated, and it includes all the children who undergo mandatory vaccination, plus everybody who doesn't, but benefits by herd immunity.

And it's with that benefit of a proven medical treatment weighed against the costs of indemnification that I think it stands. I don't see that in xenotransplantation. We have the impediment, but we don't have any evidence yet for clinical benefit.

DR. SYKES: But would those vaccines have gone into clinical trials if people knew in advance that somebody was going to try to make a connection between thimerosal and autism?

DR. ROTROSEN: They would—yes, they probably would have, maybe with government sponsorship as opposed to industry in some cases, but the difference here I think still comes down to there's great precedent; there's dozens of vaccines that work. We don't have a xenotransplantation procedure yet that works.

DR. SYKES: We never will if the industry—

DR. VANDERPOOL: My concern about this is that what you would save, think of what you would lose. I mean, people aren't suable for liability for zoonotic infections?

DR. LUBINIECKI This is Tony. Could I make a comment?

HAROLD VANDERPOOL: Yes.

DR. LUBINIECKI: It's certainly true that one of the singular examples of this indemnification issue is the childhood vaccine industry, but I would point out that between when the vaccine industry got going in a major way in the '50s and '60s until when the indemnification came about in the late '80s and early '90s, the industry had shrunk from over 20 companies to four companies, and liability was certainly a major reason why that happened.

But perhaps more to the point, what might be a better way to approach number nine is rather than to recommend that a specific approach be developed, perhaps just to raise it as an issue that may be beyond the scope of this report, but that the Secretary might wish to constitute other bodies to return to visit this issue specifically in the future. Because I think it is a problem; I don't know that we're necessarily constituted to recommend how to solve it. But if history, and vaccine history as an example, it may very well be part of this particular advance of xenotourism if it succeeds.

DR. SPIRA: Let me just add a comment on that example of the vaccine indemnification program for adverse events. That also pays for itself. There's no outside money that funds that program, which is, I think, very different from this situation. When you present a protocol on xenotransplantation to a patient, what are you going to tell them? You know, for most protocols where you don't have a rich company which is willing to indemnify or force to indemnify for adverse events through clinical trial, you tell them that they're going to have to assume these risks themselves, basically. And if they can prove negligence and they can sue someone and there's someone to collect from, and I think already in these cases we've seen a lot of companies go out of the business of supporting these transplants, there's not going to be anyone to sue.

So removing this and putting it more on the patient, which is where I think it is already, I don't think accomplishes very much.

DR. VANDERPOOL: Robyn, do you have a final issue to raise? We need to talk before we leave about what we're going to do.

MS. SHAPIRO: Well, I favor the previous comment, where the suggestion is we—I doubt that—first of all, we don't address this in the report at all. We're just kind of coming out with it directly, anyway. So to come up with an answer to an acknowledged problem in a one-sentence recommendation which has huge implications, I think is overly ambitious. So I would rather see a flag of the issue, as was suggested, and how this may be something that should be thought out.

DR. SYKES: I agree with that.

DR. VANDERPOOL: Bob or Tony, do you have issues you would like to raise on the report yourselves?

DR. MENDEZ: I don't think so now. I did just mention a few things that Mary will get tomorrow about part of that stem cell part there, and just to delete the embryo—the word "embryo" so as to not cause so much possible problems.

DR. LUBINIECKI: This is Tony. I have three brief comments. One, it occurs in the text as well as in the recommendations on all three points. On the xenotourism point, and also in recommendation number 12, it repeatedly talks about U.S. citizens. And I think U.S. citizens are certainly important, but I think what we're really trying to discuss is U.S. residents as opposed to U.S. citizens. It's my understanding that U.S. residents may also travel abroad and return to the U.S.

DR. VANDERPOOL: Okay.

DR. LUBINIECKI: The second relates to the funding chapter, as well as, I think it's recommendation number seven. In recommendation number seven we talk about an industrial-academic partnership, but I think if we're going to recommend something about that, it should be a government-industrial-academic alliance or partnership. I think the government does not figure in number seven in any way, and I think that perhaps should be included.

But more to the point, back in the funding chapter there are three paragraphs, and when I look at it, I see the first paragraph as essentially discussing matters of fact, and the second two paragraphs dealing largely with opinions. And one way perhaps to resolve the discussion we had a bit earlier might be to in fact delete the second two paragraphs and just stick with the first paragraph, and then basically include it with a statement that says we basically have a funding shortage that affects the current rate of progress, and just leave it at that. That's just as a suggestion.

The third comment is in the area of animals. And I would certainly defer to Alan and Mike's opinion, but I think we could certainly say two things about animal usage in the report. And the first is that in general, for both research application and eventually for therapeutic use, that the animals must be treated humanely and must be treated with the respect due to their participation in activities designed to save human life.

And the second is that issues, ethical issues, may arise, but it's a little difficult to deal with them at this time, because we do not as yet have a specific embodiment of this technology that we know we want to employ, and that further consideration will be due once that specific embodiment is created.

So at any rate, I would offer that thought to the group, and I think perhaps it might also be worth repeating that in the recommendations, that that's an unfinished consideration that will be re visited in the future.

DR. VANDERPOOL: Okay. Thank you. I recommend that we all send to Mary or give to Mary, maybe fax to Mary all our suggested emendations and word changes. I think she and our editors have a good idea of what the basic changes would be.

And Mary, what are some of your thoughts about time frame at this point for this report?

DR. GROESCH: Well, I think we all agree that we need to get this finalized in terms of being ready for public comment as soon as possible, so if we could get your comments in over the next, say, week or so, then we could try to turn around another version of it.

And I can send the paper, too, electronically as well if that would be helpful. I think it would. And then I think we need to figure out whether we want—we still have a number of issues in our comments table that need to be gone through, and some of you may be able to address them in the comments that you send in, but I think we're going to have to have more discussion. And whether you want to have a face-to-face meeting where all we discuss are the documents and not have other presentations, just focus on that, or have it by teleconference, we can try and arrange that as well.

So I would like to know what the group's preference is for doing this.

DR. SYKES: I think it could be done by teleconference.

DR. SCHECKLER: If you want to do it fast, you better do it by teleconference.

DR. GROESCH: Well, we will have to—just a comment that John made as he left, was to the effect that now both of these draft reports have left their respective drafting work groups and they are reports of the entire committee, so these are discussions of the full committee. And this is a federal advisory committee, and we can't have discussions of the full committee kind of off-line. So we can have a public teleconference, but we can't set that up at the drop of a hat. We do need to advertise it in the *Federal Register*. So it will take a little bit of time to set up. Not ages, but we do have to go through formal procedures for it. But that's certainly a mechanism that we can use.

DR. MICHAELS: Could we see what kind of comments you get within the next week, and then when you see that, if it's something that we could do as a teleconference, even a public forum teleconference, that might be easiest. But if it really looks like we need a face-to-face, you can let us know.

DR. VANDERPOOL: That seems excellent to me, Marian. I think we have to turn our comments and suggestions, particular ones we have, either give them to Mary as you walk out of the room or put them in the mail within two days. I mean, it's got to be done, and it's got to be done soon, and otherwise they get lost in our stacks on our desk.

And the other thing is, Mary, you stay in contact with us and we will stay in contact, you and I, and we will make proposals to the membership as to whether we can do this—whether we have all the changes we need, which I think they can work off the transcript, significantly.

Feel free to contact us committee members about editorial changes; I mean, to send both documents to everyone and say, please, take an hour, go through this. What do you think. These contain the suggestions we all agreed to, or at least appeared to agree to in our meeting.

So I would really, really like for us to have these documents in—I would like to see the "Informed Consent" as more or less a final document within two weeks, and this document to be ready to look at again and review within two weeks.

DR. SYKES: Harold, do you think this needs to be done with handwritten comments? I mean, I have at least 40 or 50 that I've written in, and my handwriting is awful, so my preference would be to type them in the file with marked changes. But can you work with that?

DR. VANDERPOOL: Sure. You can't read my handwriting either, Megan. So I would just as soon do it via e-mail, too. But if you have a legible hand and you want to give your things to Mary now, do so right now.

DR. GROESCH: But I will send them out electronically, and you can work in track changes and send them in to us. Yes, I will send both of them.

DR. VANDERPOOL: And we are all committed to doing this soon. Marian?

DR. MICHAELS: Mary, do you want it in track changes from everybody, or do you want it more the way they have here, page X, line 21 to 24, make this change? Because if you do track changes from all of us, someone is going to have a heck of a time. So maybe the way—

MS. SHUMAN: It's no problem. Whatever is your preference, we'll work with whatever is easiest for you to do.

Agenda Item: Closing Remarks

DR. VANDERPOOL: It's been great. Let's have—it's been a great time together, and I really look forward to having a sense of accomplishment and completion with these two reports. And I just hope that at some point we will all, some way or another, in our imaginations, at least, be in the same room together again.

DR. GROESCH: And I just want to make one clarification. I will send a note around about this, but I've gotten a couple of questions. Because we had our dinner last night and we presented some certificates of service to

members, it doesn't mean that you're finished. I just wasn't certain whether we would have another face-to-face meeting, so we wanted to be sure to get together and do that. But you are still members. Whether you were appointed for one, two, or three years and received a certificate last night, you are still members, you have been extended, and you will still be members until we name other members.

So I just want you to know, your job is not done just because we had a dinner last night. Okay?

DR. VANDERPOOL: Thank you. Thank you.